Analysis of polymorphisms in EGF, EGFR and HER2 genes in pancreatic neuroendocrine tumors (PNETs)

Short title: EGF, EGFR and HER2 SNPs in pancreatic NETs

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ABSTRACT

Objectives: Pancreatic neuroendocrine tumors (NETs) are rare and account for about 7% of all cancers occurring in the pancreas. The epidermal growth factor family of receptors and their ligands play an important role in the growth and progression of tumors but their role in PNET development remains unknown. We hypothesized that functional single nucleotide polymorphisms (SNPs) in the *EGF*, *EGFR*, and *HER2* genes might affect individual susceptibility to PNETs development and invasion like it was shown for various other tumors.

Methods: We genotyped 68 patients with unresectable PNETs and 300 controls to evaluate the association between *EGF*, *EGFR*, and *HER2* polymorphisms and susceptibility to PNETs and presence of metastases.

Results: Genotype analysis of three SNPs *EGF +61A/G* (rs4444903), *EGFR +1562 G/A* (rs11543848), and *HER2 +1963 A/G* (rs1136201) showed that carriers of *EGFR* +1562 AG genotype and AA/AG *EGF*+61/*HER2*+1963 genotype combination are at risk of developing PNET. Furthermore, EGFR +1562 AA genotype could be associated with the susceptibility to insulinoma development.

Conclusions: Our results suggest involvement of EGFR signaling pathway in etiology of PNET development.

Keywords: pancreatic neuroendocrine tumors, *EGF*, *EGFR*, *HER2*, metastasis

Introduction

Pancreatic neuroendocrine tumors (PNETs) are a heterogeneous group of rare neoplasms that originate from pancreatic endocrine tissue and range from quite indolent to highly aggressive[1]. Functional PNETs (PNET-F) produce specific hormones and hormone-related syndromes while nonfunctional PNETs (PNET-NF) instead cause morbidity and mortality by invading normal tissues[2]. The frequency of both functional and nonfunctional PNETs is on the continuous rise[3] and over 60% of tumors are diagnosed at the advanced stages, with metastases present[4]. The only effective approach is surgical resection, which is possible in 15% of PNET patients[5] while other treatments for patients with the advanced disease include therapy for the relief of clinical symptoms and tumor growth stabilization[6]. While the genetics of a small proportion of inherited forms of PNETs is better understood, little is known about the oncogenesis of sporadic PNETs, which form the tumor majority[7, 8]. In recent years, several molecular profiling studies have revealed important PNET-signature genes and documented a strong statistical association between common genetic variations and genetic susceptibility to PNETs[9, 10]. Interestingly, many of these variants are not correlated with protein-coding changes sites suggesting that they rather play a role in gene regulation[11, 12].

In homeostasis, growth factors and their receptors have a function in the regulation of cell proliferation, differentiation, adhesion, and migration[13]. However, in pathological settings, they can become drivers of tumorigenesis[14], migration and invasion[15]. Inappropriate activation or overexpression of epidermal growth factor (EGF) and its epidermal growth factor receptor (EGFR) is frequently present in various tumors[16-19]. The binding of EGF polypeptide induces EGFR homodimerization or heterodimerization with human epidermal growth factor receptor 2 (HER2, ERBB2), and subsequent activation of downstream RAS-RAF-MAPK signaling pathway which, in tumor settings, has been associated with the growth and progression of neoplasia[20]. Expression of EGFR and HER2 has been identified in most

of the PNETs[21-23] and next-generation sequencing analysis showed that EGFR and HER2 have missense genomic alterations or amplification in neuroendocrine neoplasms[24]. Indeed, it has been previously demonstrated that activated EGFR expression correlates with tumor growth[25], progression, and worse prognosis in PNET patients[26].

The expression and activity of both EGF and its receptors EGFR and HER2 can be modified by several known polymorphisms in their genes[27-29]. One of the most studied *EGF* polymorphisms is +61A/G (rs4444903) that has been associated with a higher EGF levels and higher susceptibility to various carcinomas in individuals with variant alleles[30]. Similarly, in *HER2* SNP +1963 A/G (rs1136201) presence of variant allele has been associated with a higher risk of breast cancer[31] and worse survival in patients with advanced cancer of the head and neck[27]. For *EGFR*, it has been demonstrated that lysine substitution in polymorphism +1562 G/A (R521K) (rs11543848) is associated with lower EGFR expression and lower risk of the lung[32] and breast[31] cancer development.

Given that the epidermal growth factor family and their ligands play a central role in the regulation of cell growth, proliferation, and migration, the study of polymorphisms affecting their expression may address their relationship with the occurrence of the disease and its progression. Based on previous findings, in this research, we decided to investigate the correlation between *EGF* rs4444903, *EGFR* rs11543848, and *HER2* rs1136201 and susceptibility to PNETs development. Moreover, we investigated the potential correlation with polymorphisms of the studied genes and presence or absence of metastasis in patients with functional and nonfunctional PNET forms.

Patients and Methods

Patients

The study included 68 patients diagnosed with pancreatic neuroendocrine tumors and 300 healthy unrelated individuals. Patients, as well as controls, were Caucasians of Croatian nationality. Patients were recruited from the Department of Endocrinology, Diabetes, and Metabolism, University Hospital Centre "Sestre milosrdnice" and all gave written informed consent for the participation in this study. Diagnosis was confirmed by standard procedures including imaging techniques and endoscopic procedures followed by tumor tissue biopsies. Prior to analysis, we excluded 14 PNET cases because of clinical or genetic diagnoses of *MEN1, MEN2* or *NF1* mutation. Among patients 43 had nonfunctional and 25 functional tumors, associated with hypoglicemia[33] and diarrhea (VIP-oma [34], gastrinoma[35], glucagonoma and carcinoid syndrome[36]) respectively. Functional tumors were then further divided into PNET group consisting of patients with VIP-oma, gastrinoma, glucagonoma and carcinoid syndrome while insulinoma cases were separated from analysis of other functional PNETs due to different clinical behavior and prognosis. In 23 of 68 PNET cases, lymph node, liver, or spleen metastases were present when the primary tumor was detected. The study was approved by Ethics Committees of the University Hospital Centre "Sestre Milosrdnice" and the School of Medicine University of Zagreb. DNAs from 300 healthy volunteers were obtained from the Croatian Tumor and DNA Bank for Basic Research[37].

Methods

SNP genotyping. The analyzed SNPs included *EGF* rs4444903, *EGFR* rs11543848, and *HER2* rs1136201. Genomic DNAs were isolated from peripheral blood of patients and healthy controls using proteinase K digestion and phenol-chloroform extraction. SNP genotypes were determined using predeveloped TaqMan® SNP Genotyping Assays C_27031637_30 for rs4444903, C_16170352_20 for rs2227983, and C_7452451_1_ for rs1136201 using the Applied Biosystems 7300 Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's standard protocol. For quality control, 15% of randomly

selected samples of both cases and control were analyzed a second time, without finding any discrepancies. Control samples covering three possible SNP genotypes and no template control were run in parallel with tested samples in each experiment.

Statistics

The odds ratio (OR) and 95% confidence intervals, as well as $\chi 2$ and Fisher's test, were calculated for *EGF*, *EGFR* and *HER2* polymorphisms and PNET risk by using GraphPad (GraphPad Software, San Diego, CA). The P values are all two-sided and the level of significance was 0.05.

Results

Blood samples of 68 patients with pancreatic neuroendocrine tumors and 300 healthy cancer-free unrelated individuals were included in the study. For all polymorphisms, observed genotype distributions were in Hardy-Weinberg equilibrium in both the controls and PNETs. Data on patients' clinical characteristics are presented in Table 1.

Genotype distribution of *EGF* +61A/G, *EGFR* +1562 G/A (R521K), and *HER2* +1963 A/G (I655V) polymorphisms among PNET patients and healthy controls is presented in Table 2. Multiple logistic regression analysis revealed no association between *EGF* and *HER2* polymorphism genotype variants and the risk of PNET development ($p>0.05$; Table 2). However, we found statistically significant association between presence of *EGFR* +1562 AG genotype and risk of PNET development ($p=0.045$, Table 2). Even though *EGF* and *HER* polymorphisms did not show any effect on PNET development on their own, there was a statistically significant association between AA/AG *EGF*+61/*HER2*+1963 genotype combination and risk of PNET development ($p=0.038$; Supplementary Table 1). Supplementary Tables 1-4 show population at risk for developing PNETs according to various *EGF*, *EGFR* and *HER2* genotype combinations.

Distribution differences in *EGF* +61A/G, *EGFR* +1562 G/A (R521K), and *HER2* +1963 A/G (I655V) genotypes divided between patients with nonfunctional and functional PNETs in comparison to healthy controls are given in Table 3 and Table 4, respectively. There were no statistically significant associations between the analyzed SNPs and the risk of developing either functional or nonfunctional PNETs (Table 3, Table 4). Likewise, separate analysis of genotype distribution among insulinoma patients and healthy controls showed no statistically significant association between above mentioned *EGF*, *EGFR*, or *HER2* polymorphism genotype variants and the risk of PNET development (Table 5).

In Table 6, distribution differences of three SNPs between patients with functional and nonfunctional PNETs are given. Even though there were no statistically significant differences between the patients with functional and nonfunctional PNETs in any of the analyzed SNP, when SNP frequencies were compared between patients with insulinoma and nonfunctional PNETs, the analysis revealed higher proportion of *EGFR* +1562 AG and AG+GG carriers in the patients with nonfunctional PNET in comparison to insulinoma patients (p=0.02 and p=0.05, Table 7).

From all PNET patients, 25 patients had metastases present at the time of diagnosis. Our analyses concerning the distribution of SNPs prevalence of these genotypes in individuals with metastases showed that there was no association between the prevalence of studied genotypes and presence or absence of metastases in our PNET patients (Table 8).

Discussion

Several research advances have been made in the field of neuroendocrine neoplasms. Despite broad research, molecular pathways that play a role in development and the genetic susceptibility in the population of patients with sporadic NETs still remain unknown[38].

Although gene mutations were confirmed in a proportion of sporadic PNETs, they are responsible for tumorigenesis of less than half of PNETs[1], while for the rest of tumors more important drivers of malignant transformation seem to be related to genetic differences between patients. *EGF* +61A>G (rs4444903), *EGFR* +1562 G>A (rs11543848) and *HER2* +1963 A>G (rs1136201) have recently been proposed to play a role in carcinogenesis and impact susceptibility to various carcinomas. In addition, EGFR overexpression has been correlated with aggressive growth in gastrinoma, subtype of functional PNET[39]. Therefore, in this study, we tried to address the potential relation of polymorphisms in EGFR pathway with differences in incidence and metastatic status of functional and nonfunctional PNETs. Similar like in the other tumors, we found that SNPs in EGFR signaling pathway could be associated with an increased risk of PNET development. We observed a higher prevalence of the AG genotype of *EGFR +1562* SNP in the PNET patients in comparison to control group which goes in line with previous observations that wild type allele G predisposes to higher risk of lung and breast cancer development[31, 32]. Moreover, combined AA and AG genotypes of *EGF+61* and *HER2+1963* were associated with the risk of PNET development which is partially in contrast with previous studies that reported *EGF*+61 G allele as a risk factor for gastric cancer and glioma susceptibility[40, 41]. In contrast, we found that none of the variant genotypes of neither *EGF, EGFR,* nor *HER2* is associated with the presence of the metastasis.

The role of the epidermal growth factor family of receptor tyrosine kinases has been established in tumorigenesis of different tumors, including gastrointestinal and pancreatic cancer[42-45]. Several studies have suggested that EGFR signal transduction pathway could be targeted for a therapy of unresectable metastatic gastrointestinal carcinoid tumors and pancreatic endocrine tumors[26, 46] since EGFR pathway activation can be detected in a large proportion of PNETs with high grading and poor prognosis[22, 26]. However, trials with EGFR tyrosine kinase inhibitors resulted in low tumor response [47] which could be partially explained by the fact

that EGFR can be detected in a large proportion of gastrinomas but much less so in insulinomas and nonfunctional PNETs[22]. Therefore, we decided to divide the PNET group into functional and nonfunctional patients. Given that previous publications showed that insulinoma PNETs are distinct tumor subtype that has different clinical behavior and prognosis[3] we decided to separated them from other functional PNETs. With this stratified analysis we showed higher presence of *EGFR* +1562 AG genotype and combined AG+GG genotype in patients with nonfunctional PNET in comparison to insulinoma patients suggesting that presence of AA genotype could be associated with the risk of insulinoma development. Interestingly, other authors have also showed that the molecular alterations in sporadic insulinoma are quite different from that in non-insulinoma PNETs. Cao et al. identified recurrent YY1 T372R mutation in insulinomas[48], however this was not found in other PNETs[49].

PNETs tend to show an aggressive course with metastases to the lymph nodes, liver, or spleen. Despite significant advances in treatment options, the presence or development of liver metastases is a poor prognostic factor for survival of PNET patients. For many years, the only known mutation responsible for aggressive growth and metastasis in these tumors was in MEN1 gene. However, in 2011. Jiao et al. reported frequent alterations in variety of genes in the mTOR pathway that are associated with metastasis and proliferation in PNETs[50]. Since EGFR and HER, together with numerous growth factors, are a part of phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR pathway we decided to investigate if above mentioned genetic polymorphisms are associated with increased risk of metastases in patients with PNET. However, in our analysis none of the targeted SNPs in investigated genes were significantly associated with the presence of metastases.

In conclusion, our study is to the best of our knowledge the first to investigate the relationship between the potential role of *EGF, EGFR,* and *HER2* polymorphisms and PNET susceptibility and metastasis presence. Our results showed statistically significant association between the

EGFR +1562 AG and AA/AG *EGF*+61/*HER2*+1963 genotype combination and susceptibility to PNET development. These results suggest involvement of EGFR signaling pathway in PNET development. The main limitation of our current study is the relatively small number of functional, but also overall PNET patients, however since this is a rare neoplasm, this problem is difficult to address. In addition, it is possible that either other metastasis-related genes or other SNPs in *EGR*, *EGFR*, and *HER2* genes might also contribute to disease and metastasis development. Therefore, further investigations are needed to better understand the genetic basis for PNET development, particularly in aggressive tumor subtypes with present metastases.

DECLARATIONS

Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and informed consent to participate

Written informed consent was obtained from all patients included in the study. The study was approved by the ethics committee of University Hospital Centre "Sestre Milosrdnice", Zagreb and Medical School, University of Zagreb, and was performed under the ethical standards of the Helsinki Declaration.

Authors' contributions

S. Marinović: Investigation, Formal analysis, Writing - Original Draft, **M. Cigrovski Berković**: Resources, Investigation, Writing- Original Draft **V. Zjačić-Rotkvić:** Resources, Funding Acquisition **S. Kapitanović**: Conceptualization, Funding Acquisition, Writing-Reviewing and Editing.

Financial support: The present study was supported by the grants of Croatian Science Foundation (grant number HRZZ-IP-2016-06-1430) and the Ministry of Science, Education, and Sports of the Republic of Croatia (grant numbers 134-1342428-0491 and 098-0982464-2508)

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Table 1 Characteristics of pancreatic neuroendocrine tumor patients

	Pancreatic neuroendocrine tumor cases	Healthy controls
Number (n)	68	300
Mean age, years (range)	55.22 (22-86)	64.03 (26-88)
Gender (n)		
Male	29	171
Female	39	129
Tumor functional status (n)		
Functional	25	
Nonfunctional	43	
Metastasis present		
Yes	23	
No	45	

Table 2 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K) and *HER2* +1963 A/G (I655V) polymorphisms in pancreatic neuroendocrine tumor patients (excluding insulinoma) and healthy controls

Polymorphisms		PNET 55 (%)	Controls 300 (%)	OR (95% CI)	P
EGF +61 A/G	AA	16 (29,1)	108 (36)	1	
	AG	31 (56,4)	134 (44.7)	0.640 (0.332-1.232)	0.200
	GG	8 (14,5)	58 (19.3)	1.074 (0.433-2.660)	>0.999
	AG + GG	39 (70,1)	192 (64)	0.729 (0.389-1.367)	0.110
	A	63 (57,3)	350 (58.3)	1	
	G	47 (42,7)	250 (41.7)	0.957 (0.634-1.445)	0.833
EGFR +1562 G/A (R521K)	AA	0	17 (5.7)	1	
	AG	28 (50,9)	118 (39.3)	0.118 (0.006-2.036)	**0.045**
	GG	27 (49,1)	165 (55)	0.171 (0.010-2.945)	0.136
	AG + GG	55 (100)	283 (94.3)	0.145 (0.008-2.465)	0.086
	A	28 (25,4)	152 (25.3)	1	
	G	82 (74,6)	448 (74.7)	1.006 (0.631-1.605)	>0.999
HER2 +1963 A/G (I655V)	AA	42 (76,3)	197 (65.7)	1	
	AG	11 (20)	91 (30.3)	1.764 (0.868-3.584)	0.141
	GG	2 (3,7)	12 (4)	1.279 (0.275-5.931)	0.999
	AG + GG	13 (23,7)	103 (34.3)	1.689 (0.867-3.289)	0.157
	A	95 (86,4)	485 (80.8)	1	
	G	15 (13,6)	115 (19.2)	1.502 (0.839-2.686)	0.182

Table 3 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K), and *HER2* +1963 A/G (I655V) polymorphisms in nonfunctional pancreatic neuroendocrine tumor patients and healthy controls

Polymorphisms		PNET NF 43 (%)	Controls 300 (%)	OR (95% CI)	P
EGF +61 A/G	AA	12 (27.9)	108 (36)	1	
	AG	24 (55.8)	134 (44.7)	0.620 (0.300-1.286)	0.213
	GG	7 (16.3)	58 (19.3)	0.920 (0.351-2.368)	>0.999
	AG + GG	31 (72.1)	192 (64)	0.688 (0.349-1.393)	0.393
	A	48 (55.8)	350 (58.3)	1	
	G	38 (44.2)	250 (41.7)	0.902 (0.572-1.410)	0.726
EGFR +1562 G/A (R521K)	AA	0 (0)	17 (5.7)	1	
	AG	21 (48.8)	118 (39.3)	0.312 (0.028-1.773)	0.477
	GG	22 (51.2)	165 (55)	0.416 (0.038-2.737)	0.702
	AG + GG	43 (100)	283 (94.3)	0.365 (0.034-2.198)	0.487
	A	21 (24.4)	152 (25.3)	1	
	G	65 (75.6)	448 (74.7)	0.952 (0.561-1.607)	0.895
HER2 +1963 A/G (I655V)	AA	31 (72.1)	197 (65.7)	1	
	AG	10 (23.2)	91 (30.3)	1.432 (0.696-2.918)	0.469
	GG	2 (4.7)	12 (4)	0.944 (0.216-4.406)	1.000
	AG + GG	12 (27.9)	103 (34.3)	1.351 (0.665-2.660)	0.491
	A	72 (83.7)	485 (80.8)	1	
	G	14 (16.3)	115 (19.2)	1.219 (0.681-2.219)	0.658

Table 4 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K) and *HER2* +1963 A/G (I655V) polymorphisms in functional pancreatic neuroendocrine tumor patients and healthy controls

Polymorphisms		PNET F 12 (%)	Controls 300 (%)	OR (95% CI)	P
EGF +61 A/G	AA	4 (33,3)	108 (36)	1	
	AG	7 (58,3)	134 (44.7)	0.709 (0.202-2.486)	0.534
	GG	1 (8,4)	58 (19.3)	2.148 (0.234-19.68)	0.207
	AG + GG	8 (66,7)	192 (64)	0.888 (0.261-3.021)	>0.999
	A	15 (62,5)	350 (58.3)	1	
	G	9 (37,5)	250 (41.7)	1.190 (0.512-2.764)	0.830
EGFR +1562 G/A (R521K)	AA	0 (0)	17 (5.7)	1	
	AG	5 (41,7)	118 (39.3)	0.610 (0.032-11.53)	>0.999
	GG	7 (58,3)	165 (55)	0.630 (0.034-11.52)	>0.999
	AG + GG	12 (100)	283 (94.3)	0.648 (0.036-11.41)	>0.999
	A	5 (20,8)	152 (25.3)	1	
	G	19 (79,2)	448 (74.7)	0.775 (0.284-2.113)	0.811
HER2 +1963 A/G (I655V)	AA	10 (83,3)	197 (65.7)	1	
	AG	2 (16,7)	91 (30.3)	2.310 (0.495-10.76)	0.353
	GG	0 (0)	12 (4)	1.329 (0.073-24.03)	>0.999
	AG + GG	2 (16,7)	103 (34.3)	2.614 (0.562-12.16)	0.349
	A	22 (91,7)	485 (80.8)	1	
	G	2 (8,3)	115 (19.2)	2.608 (0.604-11.25)	0.284

Table 5 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K) and *HER2* +1963 A/G (I655V) polymorphisms in insulinoma patients and healthy controls

Polymorphisms		Insulinoma 13 (%)	Controls 300 (%)	OR (95% CI)	P
EGF +61 A/G	AA	4 (30,8)	108 (36)	1	
	AG	9 (69,2)	134 (44.7)	0.551 (0.165-1.840)	0.399
	GG	0 (14,5)	58 (19.3)	4.853 (0.256-91.76)	0.300
	AG + GG	9 (69,2)	192 (64)	0.790 (0.237-2.627)	0.777
	A	17 (65,4)	350 (58.3)	1	
	G	9 (34,6)	250 (41.7)	1.349 (0.591-3.077)	0.545
EGFR +1562 G/A (R521K)	AA	2 (15,4)	17 (5.7)	1	
	AG	2 (15,4)	118 (39.3)	6.941 (0.915-52.61)	0.090
	GG	9 (69,2)	165 (55)	2.157 (0.430-10.81)	0.296
	AG + GG	11 (84,6)	283 (94.3)	3.027 (0.620-14.76)	0.1826
	A	6 (23)	152 (25.3)	1	
	G	20 (77)	448 (74.7)	0.884 (0.348-2.243)	>0.999
HER2 +1963 A/G (I655V)	AA	6 (46,1)	197 (65.7)	1	
	AG	7 (53,9)	91 (30.3)	0.395 (0.129-1.212)	0.128
	GG	0 (0)	12 (4)	0.822 (0.043-15.46)	>0.999
	AG + GG	7 (53,9)	103 (34.3)	0.448 (0.146-1.369)	0.233
	A	19 (73,1)	485 (80.8)	1	
	G	7 (26,9)	115 (19.2)	0.643 (0.264-1.568)	0.316

Table 6 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K), and *HER2* +1963 A/G (I655V) polymorphisms in functional and nonfunctional pancreatic neuroendocrine tumor patients

Polymorphisms		PNET F 12 (%)	PNET NF 43 (%)	OR (95% CI)	P
EGF +61 A/G	AA	4 (33,3)	12 (27.9)	1	
	AG	7 (58,3)	24 (55.8)	1.143 (0.278-4.685)	>0.999
	GG	1 (8,4)	7 (16.3)	2.333 (0.215-25.26)	0.631
	AG + GG	8 (66,7)	31 (72.1)	1.292 (0.327-5.099)	0.729
	A	15 (62,5)	48 (55.8)	1	
	G	9 (37,5)	38 (44.2)	1.319 (0.520-3.343)	0.644
EGFR +1562 G/A (R521K)	AA	0 (0)	0 (0)	1	
	AG	5 (41,7)	21 (48.8)		Can not
	GG	7 (58,3)	22 (51.2)		be
	AG + GG	12 (100)	43 (100)		calculated
	A	5 (20,8)	21 (24.4)	1	
	G	19 (79,2)	65 (75.6)	0.814 (0.270-2.450)	0.729
HER2 +1963 A/G (I655V)	AA	10 (83,3)	31 (72.1)	1	
	AG	2 (16,7)	10 (23.2)	1.613 (0.301-8.633)	0.711
	GG	0 (0)	2 (4.7)	1.667 (0.073-37.61)	>0.999
	AG + GG	2 (16,7)	12 (27.9)	1.935 (0.368-10.16)	0.709
	A	22 (91,7)	72 (83.7)	1	
	G	2 (8,3)	14 (16.3)	2.139 (0.450-10.15)	0.515

Table 7 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K), and *HER2* +1963 A/G (I655V) polymorphisms in insulinoma and nonfunctional pancreatic neuroendocrine tumor patients

Polymorphisms		Insulinoma 13 (%)	PNET NF 43 (%)	OR (95% CI)	P
EGF +61 A/G	AA	4 (30,8)	12 (27.9)	1	
	AG	9 (69,2)	24 (55.8)	0.888 (0.226-3.487)	>0.999
	GG	0 (14,5)	7 (16.3)	5.400 (0.253-115.1)	0.277
	AG + GG	9 (69,2)	31 (72.1)	1.148 (0.296-4.445)	>0.999
	A	17 (65,4)	48 (55.8)	1	
	G	9 (34,6)	38 (44.2)	1.495 (0.599-3.728)	0.497
EGFR +1562 G/A (R521K)	AA	2 (15,4)	0 (0)	1	
	AG	2 (15,4)	21 (48,8)	43.00 (1.574-1175)	**0.020**
	GG	9 (69,2)	22 (51,2)	11.84 (0.517-271.0)	0.104
	AG + GG	11 (84,6)	43 (100)	18.91 (0.847-422.3)	0.050
	A	6 (23)	21 (24,4)	1	
	G	20 (77)	65 (75,6)	0.928 (0.329-2.619)	>0.999
HER2 +1963 A/G (I655V)	AA	6 (46,1)	31 (72.1)	1	
	AG	7 (53,9)	10 (23.2)	0.276 (0.075-1.018)	0.083
	GG	0 (0)	2 (4.7)	1.032 (0.044-24.13)	>0.999
	AG + GG	7 (53,9)	12 (27.9)	0.331 (0.092-1.191)	0.103
	A	19 (73,1)	72 (83.7)	1	
	G	4 (30,8)	14 (16.3)	0.923 (0.272-3.132)	>0.999

Table 8 Genotype frequencies of *EGF* +61A/G, *EGFR* +1562 G/A (R521K) and *HER2* +1963 A/G (I655V) polymorphisms in pancreatic neuroendocrine tumor patients with or without metastases

Polymorphisms		PNET with metastases 23 (%)	PNET without metastases 45 (%)	OR (95% CI)	p
EGF +61 A/G	AA	6 (26.1)	14 (31.1)	1	
	AG	15 (65.2)	25 (55.6)	0.7143 (0.2094-2.215)	0.774
	GG	2 (8.7)	6 (13.3)	1.286 (0.2285-7.622)	>0.999
	AG + GG	17 (68)	31 (68.9)	0.7815 (0.2394-2.284)	0.782
	A	27 (58.7)	53 (58.9)	1	
	G	19 (41.3)	37 (41.1)	0.9921 (0.4745-1.996)	>0.999
EGFR +1562 G/A (R521K)	AA	0 (0)	2 (4.4)	1	
	AG	7 (30.4)	21 (46.7)	1.000 (0.06818-7.718)	>0.999
	GG	16 (69.6)	22 (48.9)	0.4375 (0.03345-3.376)	0.635
	AG + GG	23 (100)	43 (95.6)	0.4583 (0.04618-4.410)	>0.999
	A	7 (15.2)	25 (27.8)	1	
	G	39 (84.8)	65 (72.2)	0.4667 (0.1849-1.126)	0.135
HER2 +1963 A/G (I655V)	AA	18 (78.3)	30 (66.7)	1	
	AG	5 (21.7)	13 (28.9)	1.560 (0.4891-4.584)	0.568
	GG	0 (0)	2 (4.4)	1.800 (0.2493-24.53)	>0.999
	AG + GG	5 (21.7)	15 (33.3)	1.810 (0.5908-5.161)	0.405
	A	41 (89.1)	73 (81.1)	1	
	G	5 (10.9)	17 (18.9)	1.910 (0.6976-4.984)	0.3256

Supplementary Table 1 *EGF* and *EGFR* genotype combination frequencies in pancreatic neuroendocrine tumor patients (excluding insulinoma) and healthy controls

Genotype combinations		**PNET N=55 (%)**	**Controls N=300 (%)**	**OR (95% CI)**	***P***
EGF	***HER2***				
+61	***+1963***				
AA	AA	14 (25,4)	71 (23,7)	1	
AA	AG	1 (1,8)	35 (11,7)	6.901 (0.871-54.65)	**0.038**
AA	GG	1 (1,8)	3 (1)	0.5915 (0.057-6.112)	0.528
AG	AA	21 (38,2)	85 (28,3)	0.7981 (0.378-1.683)	0.578
AG	AG	9 (16,4)	41 (13,7)	0.8983 (0.357-2.258)	0.816
AG	GG	1 (1,8)	7 (2,3)	1.380 (0.157-12.12)	>0.999
GG	AA	7 (12,8)	40 (13,3)	1.127 (0.420-3.023)	>0.999
GG	AG	1 (1,8)	16 (5,3)	3.155 (0.386-25.78)	0.455
GG	GG	0(0)	2 (0,7)	1.014 (0.046-22.27)	>0.999

*Fisher exact test

Supplementary Table 2 *EGF and HER2* genotype combination frequencies in pancreatic neuroendocrine tumor patients (excluding insulinoma) and healthy controls

Genotype combinations		**PNET N=55 (%)**	**Controls N=300 (%)**	**OR (95% CI)**	***P***
EGF	***EGFR***				
+61	***+1562***				
AA	AA	0 (0)	6 (2)	1	
AA	AG	10 (18,2)	42 (14)	0.311 (0.016-5.981)	0.577
AA	GG	6 (10,9)	60 (20)	0.716 (0.036-14.22)	>0.999
AG	AA	0 (0)	9 (3)	Cannot be calculated	
AG	AG	13 (23,6)	55 (18,3)	0.316 (0.016-5.970)	0.582
AG	GG	18 (32,7)	70 (23,4)	0.293 (0.015-5.448)	0.591
GG	AA	0 (0)	2 (0,7)	Cannot be calculated	
GG	AG	3 (5,5)	22 (7,3)	0.494 (0.022-10.87)	>0.999
GG	GG	5 (9,1)	34 (11,3)	0.482 (0.023-9.835)	>0.999

*Fisher exact test

Supplementary Table 3 *EGFR and HER2* genotype combination frequencies in pancreatic neuroendocrine tumor patients (excluding insulinoma) and healthy controls

Genotype combinations		**PNET N=55 (%)**	**Controls N=300 (%)**	**OR (95% CI)**	***P***
EGFR	***HER2***				
+1562	***+1963***				
AA	AA	0 (0)	14 (4,7)	1	
AA	AG	0 (0)	2 (0,6)	Cannot be calculated	
AA	GG	0 (0)	1 (0,4)	Cannot be calculated	
AG	AA	22 (40)	77 (25,7)	0.1188 (0.006-2.071)	0.068
AG	AG	4 (7,3)	40 (13,3)	0.3103 (0.015-6.131)	0.563
AG	GG	0 (0)	2 (0,6)	Cannot be calculated	
GG	AA	20 (36,4)	105 (35)	0.1775 (0.010-3.097)	0.220
GG	AG	7 (12,7)	50 (16,7)	0.2322 (0.012-4.315)	0.331
GG	GG	2 (3,6)	9 (3)	0.1310 (0.005-3.044)	0.183

*Fisher exact test

Supplementary Table 4 *EGF, EGFR and HER2* genotype combination frequencies in pancreatic neuroendocrine tumor patients (excluding insulinoma) and healthy controls

Genotype combinations			**PNET N=55 (%)**		**Controls N=300 (%)**		**OR (95% CI)**	***P***
EGF	***EGFR***	***HER2***						
+61	**+1562**	**+1963**						
AA	AA	AA	0	(0)	5	(1,7)	1	
AA	AA	AG	0	(0)	1	(0,3)	Cannot be calculated	
AA	AA	GG	0	(0)	0	(0)	Cannot be calculated	
AA	AG	AA	10	(18,2)	29	(9,7)	0.2554 (0.012-5.030)	0,573
AA	AG	AG	0	(0)	13	(4,3)	Cannot be calculated	
AA	AG	GG	0	(0)	0	(0)	Cannot be calculated	
AA	GG	AA	4	(7,3)	37	(12,3)	0.7576 (0.035-16.10)	>0.999
AA	GG	AG	1	(1,8)	21	(7)	1.303 (0.046-36.61)	>0.999
AA	GG	GG	1	(1,8)	3	(1)	0.2121 (0.006-6.822)	0.444
AG	AA	AA	0	(0)	7	(2,3)	Cannot be calculated	

AG	AA	AG	0	(0)	1	(0,3)	Cannot be calculated	
AG	AA	GG	0	(0)	1	(0,3)	Cannot be calculated	
AG	AG	AA	10	(18,2)	31	(10,3)	0.2727 (0.013-5.363)	0.570
AG	AG	AG	3	(5,5)	22	(7,3)	0.5844 (0.026-13.07)	>0.999
AG	AG	GG	0	(0)	2	(0,7)	Cannot be calculated	
AG	GG	AA	11	(20)	47	(15,7)	0.3755 (0.019-7.294)	0.576
AG	GG	AG	6	(10,9)	18	(6)	0.2587 (0.012-5.358)	0.552
AG	GG	GG	1	(1,8)	4	(1,3)	0.2727 (0.008-8.466)	>0.999
GG	AA	AA	0	(0)	2	(0,7)	Cannot be calculated	
GG	AA	AG	0	(0)	0	(0)	Cannot be calculated	
GG	AA	GG	0	(0)	0	(0)	Cannot be calculated	
GG	AG	AA	2	(3,6)	17	(5,7)	0.6364 (0.026-15.37)	>0.999
GG	AG	AG	1	(1,8)	5	(1,7)	0.3333 (0.010-10.12)	>0.999
GG	AG	GG	0	(0)	0	(0)	Cannot be calculated	
GG	GG	AA	5	(9,1)	21	(7)	0.3554 (0.016-7.455)	0.560
GG	GG	AG	0	(0)	11	(3,7)	Cannot be calculated	
GG	GG	GG	0	(0)	2	(0,7)	Cannot be calculated	

*Fisher exact test