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EXAMINATION OF INTRADUCTAL PAPILLARY MUCINOUS NEOPLASMS OF PANCREAS AND THEIR ASSOCIATION WITH EXTRAPANCREATIC MALIGNANCIES

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ISPITIVANJE INTRADUKTALNIH PAPILARNIH MUCINOZNIH NEOPLAZMI PANKREASA I NJIHOVE POVEZANOSTI SA EKSTRAPANKREASNIM MALIGNITETIMA

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To my family and to all those who were supporting me during the years

Examination of intraductal papillary mucinous neoplasms of pancreas and their association with extrapancreatic malignancies

Objectives: The primary objective of the thesis is to assess the prevalence and the incidence of extrapancreatic malignancies (EPMs) in a cohort of Italian patients diagnosed with intraductal papillary mucinous neoplasms (IMPNs) of pancreas, and to identify the risk factors for their occurrence. The secondary objective is to assess an association between pancreatic IPMN and selected single nucleotide polymorphisms (SNPs) within 8q24 region of human genome. The tertiary objective of the thesis is to assess whether patients with pancreatic IPMNs have an increased propensity to develop colorectal adenomas.

Methods: In order to meet the objectives three separate studies were conducted: the hospital-based multicentric study, the genetic association study and the colonoscopic case-control study.

The hospital-based multicentric study was conducted in Italy from January 2010 to January 2011 in eight participating centers. Three hundred ninety IPMN cases were included in the study and screened for EPMs. EPMs were grouped according to time of their diagnosis as previous, synchronous (both prevalent) and metachronous (incident). The distribution of demographics, medical history and lifestyle habits was assessed among cases. The observed/expected (O/E) ratio of prevalent EPMs was calculated.

The genetic association study was performed on 117 IPMN cases and 231 age and gender matched controls. Cases were enrolled at the Digestive Endoscopy Unit, Policlinico Agostino Gemelli, Rome, Italy with either a prevalent or incident IPMN diagnosis, diagnosed from January 2010 to June 2011. Status of selected SNPs (rs6983267, rs6993464, rs7014346, rs10505477) was determined using a StepOne Real-time PCR system (Applied Biosystems) and TaqMan SNP Genotyping Assay ™ 40X. Unconditional multiple logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of selected SNPs and IPMNs.

The colonoscopic case–control study was conducted at Catholic University and University Sapienza, Rome, Italy on 122 cases and 246 controls. Cases were patients with IPMNs without history of colorectal cancer, who underwent screening colonoscopy for the first time. Controls were individuals who underwent first time colonoscopy for screening or evaluation of non-specific abdominal symptoms. Prevalence of colon polyps and/or colorectal cancer was determined using colonoscopy findings. Chi-square and Fisher tests were used to compare the distributions of categorical variables between patients with IPMNs and control subject.

Results: Ninety-seven EPMs were diagnosed in 92 patients (23.6%) in hospital-based multicentric study. Among them 78 (80.4%) were previous, 14 (14.4%) were synchronous, and 5 (5.2%) were metachronous. O/E ratios for prevalent EPMs were significantly increased for colorectal carcinoma (2.26, CI 95%: 1.17-3.96), renal cell carcinoma (6.00, CI 95%: 2.74-11.39) and thyroid carcinoma (5.56, CI 95%: 1.80-12.96). Increased age, heavy cigarette smoking, alcohol consumption and 1st-degree family history of gastric cancer were significant risk factors for EPMs, while 1st-degree family history of colorectal carcinoma was borderline. Genetic association study showed no significant association between IPMN and SNPs rs6983267, rs6993464, rs7014346, rs10505477. In colonoscopic case-control study, colorectal polyps were found in 52 cases (42.6%) and 79 controls (32.1%) ($p<0.05$). In 29 cases (23.8%) and 57 controls (23.2%) histological examination disclosed adenomatous polyps ($p=0.90$). There was no difference between the groups in relation to presence of polyps with low-grade (19.7% vs. 19.8%, $p=0.98$) and high-grade dysplasia (4.9% vs. 4.5%, $p=0.85$).

Conclusions: We report an increased prevalence of EPMs in Italian patients with IPMN, especially for colorectal carcinoma, renal cell and thyroid cancers. A systematic surveillance of IPMN cases for such cancer types would be advised. Patients with IPMN do not have a higher prevalence of SNPs rs6983267, rs6993464, rs7014346, rs10505477 in human chromosomal region 8q24 in respect to control population. However further research is needed in order to examine if other SNPs in the region are associated with IPMN. Patients with IPMNs are not in an increased risk for development of adenomatous colorectal polyps.

Ispitivanje intraduktalnih papilarnih mucinoznih neoplazmi pankreasa i njihove povezanosti sa ekstrapankreasnim malignitetima

Ciljevi: Primarni cilj doktorske disertacije je utvrditi prevalenciju i incidenciju ekstrapankreasnih maligniteta (EPM) u kohorti italijanskih pacijenata sa intraduktalnim papilarnim mucinoznim neoplazmama (IPMN) pankreasa i identifikovati faktore rizika za njihov nastanak. Sekundarni cilj doktroske disertacije je utvrditi da li je prisustvo IPMN udruženo sa prisustvom odabranih genetskih "single-nucleotide" polimorfizama (SNP) u 8q24 regionu humanog genoma. Tercijarni cilj doktorske disertacije je utvrditi da li pacijenti sa IPMN imaju značajno više kolorektalih adenoma u odnosu na opštu populaciju.

Metode: Kako bi se ispunili navedeni ciljevi doktorske disertacije sprovedene su tri odvojene studije: multicentrična bolnička studija, studija genetske asocijacije i kolonoskopska studija slučajeva i kontrola.

Multicentrična bolnička studija sprovedena je u osam bolničkih centara u Italiji u period od janura 2010 do januara 2011. Tri stotine devedeset pacijenata sa IPMN uključeni su u studiju i ispitani na prisustvo EPM. EPM su u odnosu na vreme otkrivanja grupisani na prethodne i sinhrone (koji zajedno čine prevalentne) i metahrone. Dobijeni podatci iskorišćeni su kako bi se izračunao odnos otkrivenih i očekivanih EPM (O/E odnos). Pacijenti sa i bez EPM su upoređeni u odnosu na demografske karakteristike, životne navike i prethodnu istoriju bolesti.

Studija genetske asocijacije sprovedena je na 177 IPMN slučajeva i 231 kontrola uparenih po polu i starosti. Novotkriveni slučajevi IPMN, kao i oni koji su imali zakazane kontrolne preglede u Službi za digestivnu endoskopiju Poliklinike Agostino Gemelli, Rim, Italija u period od januara 2010 do juna 2011 uključeni su u studiju. Status odabranih SNP (rs6983267, rs6993464, rs7014346, rs10505477) određivan je uz pomoć "StepOne Real-time PCR" (Applied Biosystems) i "TaqMan SNP Genotyping Assay ™ 40X" sistema. Bezuslovni multivarijantni logisticki regresioni model je korišćen za procenu odnosa šansi (OR) i 95%-og intervala poverenja (95% CI) asocijacije odabranih SNP sa IPMN.

Kolonoskopska studija slučajeva i kontrola sprovedena je na Katoličkom Univerzitetu i na Univerzitetu "Sapienza", Rim, Italija na 122 slučaja i 246 kontrola. Slučajevi su bili pacijenti sa IPMN bez prethodne istorije kolorektalnog karcinoma, podvrgnuti kolonoskopiji po prvi put u svrhu skrininga. Kontrole su odabrane među osobama po prvi put podvrgnutim kolonoskopiji u svrhu skrininga ili ispitivanja nespecifičnih abdominalnih tegoba. Na osnovu kolonoskopskih nalaza prikupljeni su podaci o eventualnom prisustvu adenomatoznih polipa i karcinoma kolona među slučajevima i kontrolama. Hi-kvadrat i Fišerov test su korišćeni kako bi se uporedila distribicija kategoričkih varijabli.

Rezultati: U multicentričnoj bolničkoj studiji devedeset sedam EPM je otkriveno kod 92 (23.6%) pacijenta sa IPMN. Od toga 78 (80.4%) su bili prethodni, 14 (14.4%) sinhroni i 5 (5.2%) metahroni EPM. Utvrđen je značajno povišen O/E odnos za prevalentne EPM, i to za kolorektalni karcinom (2.26, CI 95%: 1.17-3.96), karcinom bubrega (6.00, CI 95%: 2.74-11.39) i karcinom štitne žlezde (5.56, CI 95%: 1.80-12.96). Starije doba, pušenje velike količine cigareta, konzumacija alkohola i postojanje prvog srodnika sa istorijom karcinoma želuca identifikovani su kao značajni faktori rizika za nastanak EPM kod pacijenta sa IPMN, dok je pozitivna porodična anamneza za kolorektalni karcinom bila granično značajna**.** Studija genetske asocijacije nije pokazala povezanost IPMN sa SNP rs6983267, rs6993464, rs7014346 i rs10505477. U kolonoskopskoj studiji slučajeva i kontrola kolorektalni polipi nađeni su kod 52 (42.6%) pacijenta sa IPMN i 79 kontrola (32.1%) (p<0.05). Kod 29 slučajeva (23.8%) i 57 kontrola (23.2%) histološko ispitivanje utvrdilo je da su u pitanju adenomatozni polipi (p=0.90). Nije bilo razlike između slučajeva i kontrola kada je u pitanju bilo prisustvo polipa sa diplazijom niskog stepena (19.7% vs. 19.8%, p=0.98), niti visokog stepena (4.9% vs. 4.5%, p=0.85).

Zaključci: Italijanski pacijenti sa IPMN imaju povišenu prevalenciju EPM, naročito kolorektalnog karcinoma, karcinoma bubrega i karcinoma štitne žlezde. Stoga bi ovi pacijenti trebalo da budu sistemski nadzirani kako bi se navedeni tumori detektovali na vreme. Pacijenti sa IPMN nemaju veću prevalenciju SNP rs6983267, rs6993464, rs7014346 i rs10505477 u humanom hromozomskom regionu 8q24 u odnosu na opštu populaciju. Potrebna su dalja istraživanja kako bi se u ispitalo da li su drugi SNP u

ovom region udruženi sa IPMN. Pacijetni sa IPMN nisu u povišenom riziku od nastanka kolorektalnih adenomatoznih polipa.

Ključne reči: pankreas, intraduktalne papilarne mucinozne neoplazme**,** ekstrapankreasni maligniteti, 8q24 region humanog genoma, kolorektalni karcinom, kolonoskopija, adenomatozni polipi

Naučna oblast: epidemiologija

Uža naučna oblast: gastroenterologija

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1. Introduction

1.1 IPMN definition and history

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are mucin-producing epithelial tumors that show a papillary architecture and are associated with dilatation of the ducts (1). This clinical entity was reported for the first time in 1980 by Ohhashi et al. (2). They reported a patient with mucin secreting cystadenocarcinoma of the pancreas, forming a fistula that drained into the common bile duct. Many similar reports followed, giving this clinical entity various names, such as diffuse intraductal papillary adenocarcinoma (3), diffuse villous adenoma (4, 5), mucinous pancreatic duct ectasia (6), intraductal cystadenocarcinoma (7), intraductal mucin-hypersecreting neoplasm (8), mucinous ductal ectasia (9), papillary adenoma (10), intraductal mucin-producing tumor (11, 12), duct-ectatic type pancreatic ductal carcinoma (13), mucin-hypersecreting tumor (14), mucous-hypersecreting tumor (15), and intraductal papillary neoplasm (16). Term IPMN was used for the first time by Sessa et al. (17) in 1994. This name was further introduced in the classification of exocrine pancreatic tumors propagated by the World Health Organization (WHO) (18) and the fascicles of the Armed Forces Institute of Pathology (19).

1.2 Histomorphological and clinical characteristics

IPMN is characterized by intraductal papillary proliferation of mucin-producing epithelial cells. The tumor usually secretes excessive amounts of mucin. The profuse secretion of mucin by IPMN results in cystic dilatation of the pancreatic ducts. Therefore IPMNs are included among the cystic tumors of the pancreas (20). Cystic tumors of the pancreas include also serous tumors (serous cystadenoma and cystadenocarcinoma), other mucinous tumors (mucinous cystic neoplasia), and solid

pseudopapillary tumors. However IPMN has certain characteristics that distinguish it from the other cystic neoplasm of the pancreas (21). It usually affects individuals 60-70 years old, and 60-70% among affected are males (22). Tumor is usually localized in pancreatic head and always communicates with pancreatic duct determining its diffuse or segmental enlargement. Tumor has no capsule and is not characterized with calcifications. Except high concentration of mucin, tumor cystic fluid is characterized by high concentration of amylase and carcinoembryonic antigen (CEA) (22).

The epithelial cells of IPMN are characterized by a wide spectrum of dysplasia, ranging from mild, intermediate and high-grade dysplasia to invasive carcinoma. Therefore IPMNs are precursors of pancreatic carcinomas and provide model of neoplastic progression from a benign intraductal tumor through increasing grades of dysplasia to invasive adenocarcinoma. Progression from adenoma to carcinoma is estimated to occur within about 5–6 years (23, 24). At present, according to international consensus guidelines from 2012, only invasive carcinoma derived from IPMN is considered malignant, while high-grade dysplasia is not considered malignant (25).

According to duct involvement IPMNs are classified into main duct IPMNs (MD-IPMNs) associated with dilation of the main pancreatic duct, branch duct IPMNs (BD-IPMNs) associated with the dilation of one of the side branches and combined type IPMN (C-IPMN) associated with dilation of both main pancreatic duct and at least one of the side branches (Figure 1).
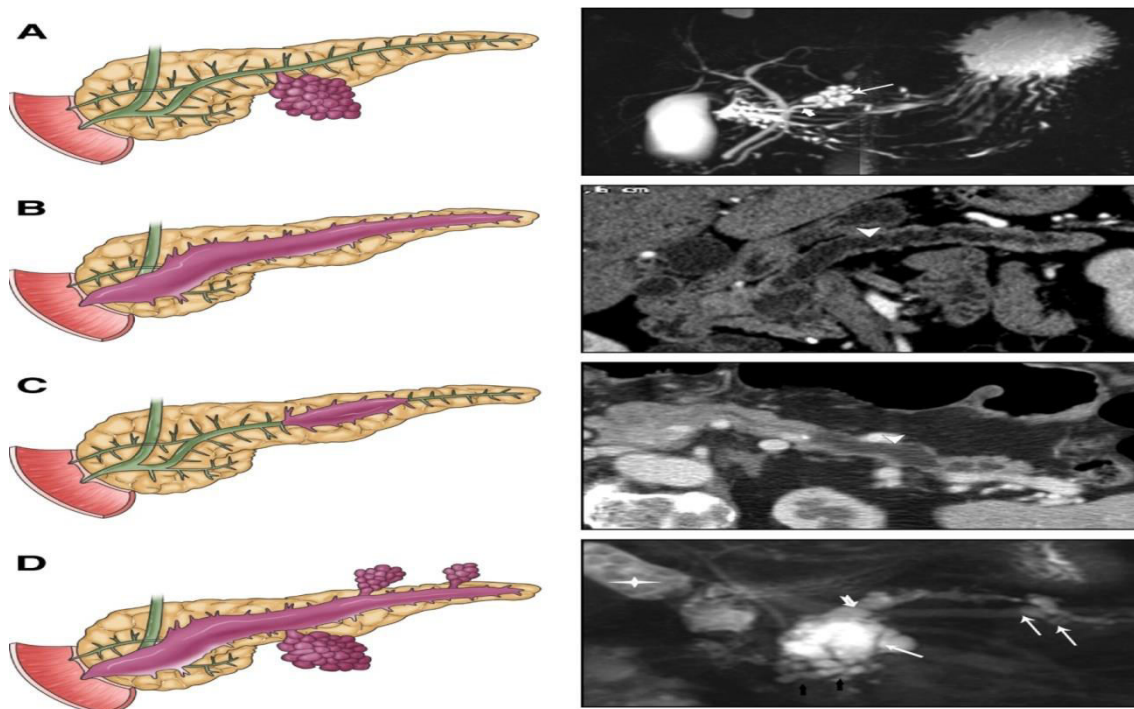
Figure 1. Morphologic classification of IPMN with schematics (left panels) and radiographic images (right panels), including (A and D) MRCP and (B and C) CT. (A) Branch duct IPMN; (B) Diffuse main duct IPMN; (C) Segmental main duct IPMN; (D) Mixed IPMN (26)

Based on the histomorphological features of papillary proliferation and the immunohistochemical characteristics of mucin glycoproteins IPMNs are classified into intestinal, pancreatobiliary, oncocytic, and gastric types (Figure 2). Intestinal type IPMN has villous papillae that express MUC2 and MUC5AC glycoproteins. Pancreatobiliary type has an intricate thin arborizing papillary structure that is consistently positive for MUC5AC and focally positive for MUC1 but not MUC2. Oncocytic type IPMN has complicated thick papillae consisting of eosinophilic oncocytic cells that are consistently positive for MUC5AC and focally positive for MUC1 and/or MUC2. Gastric type IPMN has finger-like papillary growths that are positive for MUC5AC but not for MUC1 or MUC2. The first three types originate from the main duct, whereas the gastric type usually originates from the branch ducts. It has been reported that histological subtype significantly influences the biological behavior and prognosis of IPMN (27-30). While invasive carcinoma derived from the non-intestinal type IPMN is associated with a poor prognosis (27), invasive carcinoma originating from intestinal type IPMN is often minimally invasive (29).
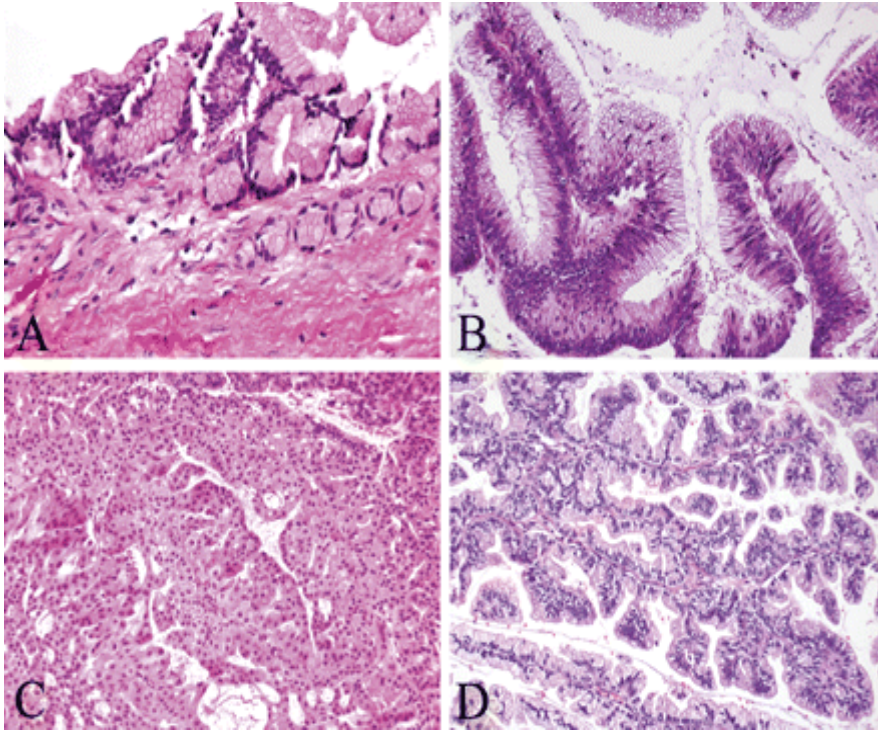
Figure 2. Epithelial subtypes of intraductal papillary mucinous neoplasm: (A) gastric; (B) intestinal; (C) oncocytic; (D) pancreatobiliary (30).

1.3. Epidemiology

Incidence of IPMN significantly increased in last decades (24, 31, 32). Study from US reported a 14-fold increase in the age- and sex-adjusted incidence of IPMN between 1985 and 2005 (31). IPMN currently accounts for 1%–3% of all exocrine pancreatic neoplasms and for 20%–50% of all cystic neoplasms of the pancreas (33-35). Furthermore large portion of IPMNs still remains clinically unrecognized, as many of them are small and asymptomatic. One imaging study reported presence of small asymptomatic IPMN in 2.8% of 2,832 consecutive outpatients examined for conditions other than known or suspected pancreatic disease (36). The percentage increased to 8.7% in individuals aged 80 years and older (36).

Since the incidence of IPMN increased in the absence of an increase in IPMN-related or overall pancreatic cancer-related mortality (31), it is likely that this increase comes from

improved IPMN diagnosis, rather than greater numbers of patients with clinically relevant disease. The diagnosis of IPMN is usually set by endoscopic retrograde cholangiopancreatography (ERCP), computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP) or endoscopic ultrasound (EUS) (Figures 3 and 4). As these imaging methods are becoming more precise and increasingly available, the possibilities for accurate IPMN diagnosis multiply. Another explanation could be found in increased awareness and better understanding of clinical, radiological, histological, and genetic aspects of IPMN. International consensus guidelines for the management of IPMN and mucinous cystic neoplasm (MCN) of the pancreas, issued for the first time in 2006 (37) and revised in 2012 (25) largely contributed to this issue.
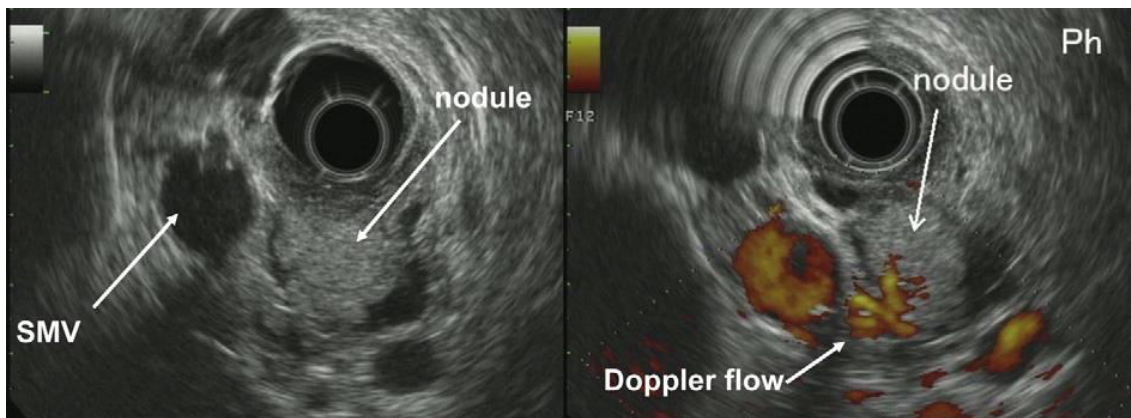
Figure 3. EUS showing a mural nodule in the dilated MPD with Doppler flow indicating the presence of a blood supply (25).
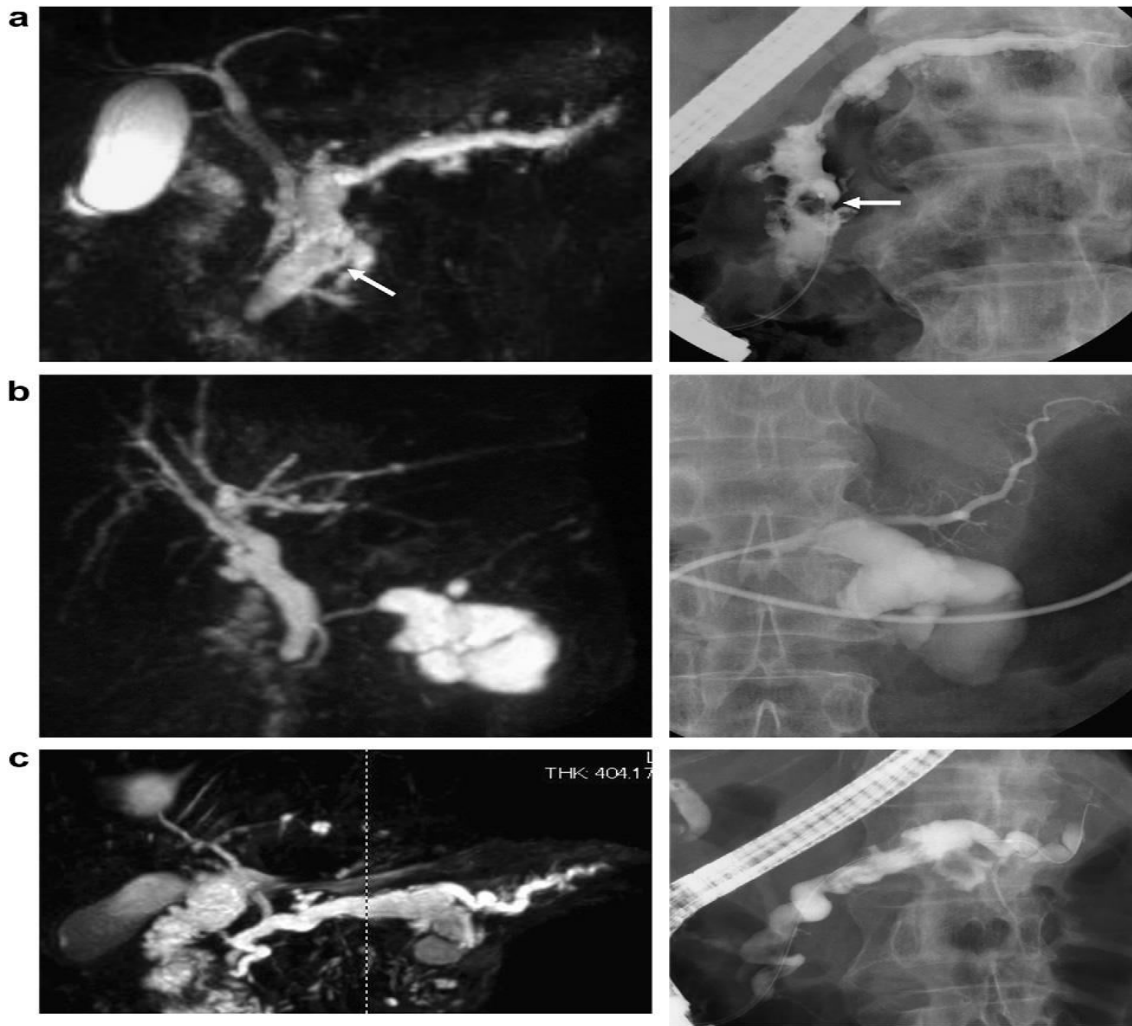
Figure 4. MRCP (left panels) and ERCP (right panels) demonstrating the three morphological types of IPMN. A. Main duct type with a mural nodule (arrows). B. Branch duct type.C. Mixed type (25).

1.4 Prognosis

IPMN has better prognosis than pancreatic ductal adenocarcinoma (PDAC), or neuroendocrine tumors of pancreas (38). Only the prognosis of patients with advanced forms of invasive IPMN is as poor as in those with PDAC (39). However MD-IPMNs and C-IPMNs at one side and BD-IPMNs on the other side differ significantly in matter of biological behavior. MD-IPMNs have been reported to have worse prognosis than BD-IPMNs in most clinical series (40). The explanation lies in greater malignant

potential of MD-IPMNs. Malignant tumor is found after resection in 57%–92% of MD-IPMNs and only 6%–46% of BD-IPMNs (37). The presence of invasive carcinoma was reported to be the strongest predictor of survival in IPMN (41-43). Five-year survival rates for patients with resected noninvasive IPMNs are 80%– 100% in compare to 40%–60% in invasive forms (24, 39, 44, 45).

1.5 Clinical presentation and management

Up to 43% of patients with IPMN are reported to be asymptomatic (40). When symptoms are present, they usually include nausea and vomiting, weight loss, back pain or abdominal pain. Some of IPMN patients may even develop exocrine and endocrine pancreatic insufficiency. As these are also the symptoms of chronic pancreatitis, IPMN can often be misdiagnosed as chronic pancreatitis. However patients with IPMN present different epidemiological characteristics than those with chronic pancreatitis. IPMN patients are more often females, are significantly older and are less frequently associated with alcohol consumption (46). Nevertheless IPMN patients may actually develop chronic pancreatitis due to obstruction of the main pancreatic duct by mucin (46).

Main therapeutic dilemma associated with IPMN is when to submit the patients to surgical resection. The therapeutic approach in IPMN patients was systematically assessed for the first time in 2006 in Senadai guidelines followed by recommendations to resect all MD-IPMNs and C-IPMNs, as well as BD-IPMNs measuring more than 3cm in diameter (37). However studies which followed after Sendai recommendations reported malignancy to be found in only 25% of resected BD-IPMNs, with invasive carcinoma in only 17.7% (25). Therefore therapeutic approach was revised in Fukuoka guidelines published in 2012, and more conservative approach for treatment of BD-IPMNs was proposed (25). The Fukuoka guidelines introduced two layers of criteria for assessment of IPMN, so called "high-risk stigmata" and "worrisome features". High risk stigmata are considered to be indicative of malignancy and include obstructive jaundice, enhanced solid component, and main pancreatic duct size $\geq$ 10 mm. Patients

with these features should undergo resection without delay (25). At the other side, worrisome features include clinical acute pancreatisis, a cyst size of ≥ 3 cm, thickened and enhanced cyst walls, nonenhanced mural nodules, main pancreatic duct size 5–9 mm, an abrupt change in the main pancreatic duct caliber with distal pancreatic atrophy, and lymphadenopathy (25). Patients with these features should be referred to EUS exam for further risk stratification (25). High resolution EUS can help in discrimination between benign and malignant IPMN by demonstrating the presence of mural nodules, irregularity and thickening of septa between cysts, and the presence of vascularity in these structures (47). Furthermore it enables fine-needle aspiration (FNA) of cystic fluid for cytological analysis (tumor or atypical cells) and laboratory tests (amylase, CEA).

The surgical procedures usually conducted for treatment of IPMN include pancreaticoduodenectomy and distal pancreatectomy. For non-resected lesions yearly follow-up is recommended for lesions with a diameter smaller than 10 mm, 6-12 months follow-up for lesions of 10–20 mm, and 3-6 months follow-up for lesions of 20–30mm (37).

1.6 Association of IPMN with extrapancreatic malignancies (EPMs)

It is acknowledged that IPMN patients have an additional risk of developing PDAC (48). Uehara et al. reported that 8% of patients with BD-IPMN developed PDAC during follow-up (49). However, number of studies reported that patients with IPMN may also be at the increased risk of harboring EPMs (Table 1). Since the first report was published in 1999 (50), several studies reported that up to 40% of IPMN patients are diagnosed with EPMs (51-61) (Table 1). Colorectal cancer (CRC) (50-54, 58, 59, 61), gastric cancer (50-54, 58, 59), lung (52, 54, 58), breast (56) and prostate cancers (60) are among the most common EPMs diagnosed in IPMN patients. Variety of other tumors has also been reported (Table 1). Increasing age (50, 52, 58, 62), positive family history for colorectal cancer (61) and presence of malignant IPMN (58) are reported as the main risk factors for EPMs among IPMNs. MUC2 expression was noticed more frequently in the IPMN coexisting with EPM than in the IPMN without EPM (63).

Some authors also suggested influence of genetic component, as IPMN was reported to be associated with familial adenomatous polyposis (FAP) syndrome (64).

Nevertheless so far available data on association between IPMN and EPM are still limited, especially in relation to European population. With exception of one study reporting on the occurrence and risk factors of EPMs among IPMNs in French population (56), all other studies were conducted in Asia (50-54, 57-59, 62) or in the United States of America (USA) (55, 60). The prognosis of IPMNs is generally favorable, with a 5-year survival rate around 60-70 % for the non- invasive forms, and 30-40 % for the invasive forms (23, 24). However, among those who develop EPMs, the prognosis is less favorable (50, 52-54). Therefore it is out of exceptional importance to investigate whether the patients with IMPN are actually at the increased risk for harboring EPMs, so they can be submitted to the increased medical surveillance including screening exams for the specific tumors.

The mechanism(s) causing the potential association between IPMN and EPMs is still unknown. It can be hypothesized that common genetic background may be responsible for observed association. Human chromosomal region 8q24 has been associated with many types of cancer (65-67). The majority of these associations lie at approximately 128 Mb on chromosome 8. Among them one prominently associated SNP, rs6983267, has been shown to interact with the myelocytomatosis viral oncogene homolog gene (MYC) (68). The region contains several other genes which could be functionally related to cancer development, including nephroblastoma over-expressed gene (NOV), which encodes a regulatory protein from the CCN family that has been associated with cancer development (69). Furthermore, as several studies suggest the possibility that some loci in 8q24 influence more than one type of cancer per locus (70) it could be a case that this region contain loci that affect general cancer susceptibility. Knowing this it can be hypothesized that presence of single-nucleotide polymorphisms (SNPs) in 8q24 region could be responsible for increased propensity of IPMN patients to develop EPMs.

1.7 IPMN and CRC

The most frequent EPM consistently found in these patients has been CRC (50-54, 58, 59, 61) (Table 1). Up to 11.9% of patients with IPMN are reported to also harbor CRC (50). CRC represents the third most common cancer worldwide with more than 1.3 million new cases (9.7% of total) and almost 700.000 deaths every year (71). Almost 55% of the cases occur in more developed regions (71), where the prognosis is relatively favourable with 5-year survival rate reaching 65% in USA, Canada, Australia and several European countries (72, 73). However, incidence is increasing in countries or areas with poor health-care resources (74), where 5-year survival is no more than 50% (75). Therefore the identification of all risk factors associated with CRC becomes of paramount importance to apply properly designed screening programs to those at higher risk than the general population in order to detect precancerous lesions or cancers at an early and more curable stage.

It is well known that almost all sporadic CRCs develop slowly over several years through the adenoma-carcinoma carcinogenetic sequence (76). Thus, it is possible to hypothesize that the increased risk of CRC in IPMN patients may be related to an increased propensity to develop colorectal adenomas, which represent the precursors of sporadic CRC. Some authors have previously reported an increased prevalence of colorectal polyps in patients with IPMN, based on which they proposed to consider screening colonoscopy for all patients with IPMN (60). This inference, however, has been based only on the result of this single study that was retrospective and based on chart review, while no data coming from studies specifically designed to prospectively assess the rate of colonic polyps in IPMN patients are available.

Table 1. Characteristics of studies conducted on association between intraductal papillary mucinous neoplasms (IPMN)s and extra-pancreatic malignancies (EPM)s and their findings in relation to EPM occurrence in general as well as in relation to specific malignancies

Author	Study design	Year	Country	No	EPM occurrence	CRC*	Gastric cancer	Lung cancer	Breast cancer	Prostate cancer	EPM risk factors
Sugiyama et al (50).	Retrospective	1999	Japan	42	35.7%	11.9%	9.5%	2.4%	2.4%	2.4%	age (p<0.05)
Osanai et al. (51)	Retrospective	2003	Japan	148	23.6%	7.4%	5.4%	3.4%	0%	1.4%	NR[γ]
Kamisawa et al. (52)	Retrospective	2005	Japan	79	35.4%	8.9%	15.2%	5.1%	2.5%	1.3%	age (p<0.05)
Choi et al. (53)	Retrospective	2006	South Korea	61	29.5%	6.6%	13.1%	0%	0%	0%	none
Eguchi et al. (54)	Retrospective	2006	Japan	69	38%	11.6%	5.8%	7.2%	0%	2.9%	age (for CRC)
Riall et al. (55)	Population-based	2007	USA	992	10.1%	2.5%	0.1%	0.8%	1.8%	1.4%	NR
Baumgaertner et al. (56)	Case-control	2008	France	178	16.8%	1.7%	NR	1.7%	5.4%	2.2%	none
Ishida et al. (57)	Retrospective	2008	Japan	61	24.6%	8%	10%	NR	NR	NR	none
Yoon et al. (58)	Retrospective	2008	South Korea	210	33.8%	7.6%	13.8%	1.4%	0%	0.5%	age invasive IPMN
Oh et al. (59)	Retrospective	2009	South Korea	37	27%	8.1%	8.1%	5.4%	0%	2.7	NR
Reid-Lombardo et al. (60)	Retrospective	2010	USA	471	40.8%	4.0%	0%	0.6%	5.1%	5.0%	NR
Lubezky et al. (61)	Retrospective	2011	Israel	82	19.5%	6.1%	0%	1.2%	3.7%	3.7%	CRC 1st-degree family history
Kawakubo et al. (62)	Prospective	2011	Japan	642	6.0%	0.9%	0.9%	0.8%	0%	0.6%	NR

*CRC=colorectal cancer

[γ]NR=not reported

2. Objectives

In the light of previously mentioned facts, three separate studies were designed in order to address potential association between IPMN and EPMs. The work hypotheses were defined as follows:

- Extrapancreatic malignancies (EPMs) occur significantly more frequent in patients with IPMNs of the pancreas in compare to the general population.
- IPMNs are associated with SNPs in 8q24 region of human genome.
- Patients with IPMN harbour significantly more colorectal adenomas than IPMN free controls.

In concordance with named hypotheses following objectives were defined:

- The primary objective of the thesis is to assess the prevalence and incidence of EPMs in a cohort of Italian patients diagnosed with IMPN, and to identify the risk factors for their occurrence.
- The secondary objective is to assess an association between IPMN and selected SNPs within 8q24 region of human genome.
- The tertiary objective of the thesis is to assess whether patients with IPMNs have an increased propensity to develop colorectal adenomas.

3. Methods

In order to meet the objectives three separate studies were conducted: the hospital-based multicentric study, the genetic-association study and colonoscopic case-control study.

3.1. The hospital-based multicentric study

The hospital-based multicentre study was conducted to assess the prevalence and incidence of EPMs in a cohort of Italian patients diagnosed with IMPN, and to identify the risk factors for their occurrence.

3.1.1 Study Design

The study was conducted at the participating centres upon Hospital Review Boards approval (77). Participating centres in Italy were: Digestive Endoscopy Unit, Università Cattolica del Sacro Cuore, and Digestive and Liver Disease Unit, "Sapienza" University, Rome; Surgery, University of Verona, and Gastroenterology, University of Verona, Verona; Gastroenterology & Gastrointestinal Endoscopy, San Raffaele Hospital, and Surgery Istituto Humanitas, Milano, Italy; Surgery, University of Pisa, Pisa; Gastroenterology, S. Agostino Hospital, Modena; Gastroenterology, Bellaria Maggiore Hospital, and Internal Medicine, University of Bologna, Bologna; Endoscopy, Ismet, Palermo (Figure 5).

Figure 5. Map of Italy with all the participating centres marked.

Prevalent cases of patients with either a new diagnosis of IPMN or those seen during follow-up at the participating units during an 18-months period (January 2010 to June 2011) were enrolled. Inclusion criteria were: a) age > 18 years old; b) will and ability to cooperate. The criteria for the identification of IPMN cases are previously described (78). Patients were excluded if they have cystic lesions other than IPMN. The type of duct involvement was determined at each participating unit by revision of clinical imaging studies and of macroscopic and microscopic examinations when available, and classified as MD-IPMNs, BD-IPMNs or C-IPMNs.

EPMs were defined as secondary primary tumour occurring in IPMN patients, with a histological confirmation. EPMs were grouped according to the time of their diagnosis: 1) previous EPMs, which were diagnosed before the IPMN diagnosis; 2) synchronous EPMs, diagnosed at the same time of IPMN; 3) metachronous EPMs, diagnosed during the follow-up of IPMN patients. We considered previous and synchronous as prevalent EPMs, while metachronous as incident EPMs. IPMN cases were followed-up for an average period of 13 months (5-31 months). The follow-up for each individual patient was scheduled according with published guidelines (37), and included abdominal

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ultrasound, CT, magnetic resonance imaging or EUS. Additional investigations, such as gastroscopy, colonoscopy or chest x-ray were performed when judged to be clinically indicated, while no standard screening procedures were performed.

3.1.2 Data collection

IPMN patients were interviewed by a trained physician who filled-in a structured questionnaire to collect data on demographics, medical and family history and lifestyle habits. To avoid possible bias due to cancer symptoms or subsequent cancer therapies (either surgical or medical), subjects were asked about lifestyle habits referred to the 12 months before the IPMN diagnosis or presentation of IPMN symptoms. The following data were recorded: age, gender, weight, height, (Body mass Index (BMI) was subsequently calculated), family history of cancers (1st and 2nd degree), clinical history (history of pancreatitis, diabetes, peptic ulcer and cholecystectomy; use of certain drugs), and lifestyle habits (cigarette smoking status and alcohol drinking assumption). Ever cigarette smokers were defined as those who ever smoked cigarettes for at least 6 months or smoked at least 100 cigarettes during the lifetime. The amount of cigarette smoking was evaluated as pack-years (number of packs per day X years of smoking). Ever alcohol drinkers were defined as those drinking at least 12.5 g of alcohol (one glass of wine, or one pint/can of beer, or one shot of hard liquor) per day for at least one year. We defined "recent onset diabetes" as diagnosed during the 12 months before the diagnosis of IPMN.

3.1.3 Statistical analysis

For previous and synchronous EPMs (prevalent EPMs), the ratio of the observed (O) number of patients with EPMs to the expected (E) number was calculated along with 95% confidence interval (CI) (79). We excluded in this calculation the metachronous tumours (incident EPMs) in view of the short follow-up. Data on age-stratified and gender-specific prevalence of cancer in Italy were used to determine the expected number of EPMs (80). We compared the distribution of the demographics, medical and family history, lifestyle habits variables among patients with and without EPMs by univariate analysis. Chi-square and Fisher's exact tests were used where appropriate.

Statistical analyses were performed using Stata 11.2 (Stata Corporation, College Station, TX).3.2.

3.2 The genetic-association study

Genetic-association study was conducted in order to assess an association between pancreatic IPMN and selected SNPs within 8q24 region of human genome.

3.2.1. Study design

The study was conducted at the Digestive Endoscopy Unit of the Catholic University Rome, Italy. Prevalent cases of patients with either a new diagnosis of IPMN or those seen during follow-up at the participating units during an 18-month period (January, 2010 to June, 2011) were enrolled. The criteria for the IPMN diagnosis have been previously described (78). The diagnosis of IPMN was considered as certain in the presence of either histological diagnosis obtained by EUS or surgical specimen, or cytologic diagnosis obtained by EUS. A highly probable diagnosis of IPMN was based on the presence of one or several main pancreatic duct and/or branch duct dilatation(s) and/or pancreatic cystic lesions communicating with pancreatic ducts at CT, magnetic resonance cholangiopancreatography with secretin stimulation (S-MRCP), ERCP or EUS. Patients were excluded if they had cystic lesions other than IPMN. The controls included patients from the same hospital with a broad range of diagnoses, enrolled during the same time period. Around 50% of the controls were outpatients, and the remaining were patients undergoing surgical interventions (laparoscopic cholecystectomy, appendicitis, inguinal hernia) or admitted for a wide spectrum of other non neoplastic diseases. Controls were matched to each IPMN case by gender and age (±5 years). Written informed consent was obtained from all study subjects. The study was conducted according to the Declaration of Helsinki and was approved by the Ethical Committee of Università Cattolica del Sacro Cuore. Both cases and controls were interviewed by trained physicians using a structured questionnaire and data on demographics, lifestyle habits (alcohol consumption, cigarette smoking), prior medical history, cancer family history and medication use were collected.

3.2.2 SNPs genotyping

SNPs rs10505477, rs6983267, rs7014346 and rs6993464 within 8q24 region of human genome have been selected to be tested in the study. Among all the SNPs within 8q24 region, these have been most frequently reported to be significantly associated with cancer susceptibility in general.

Genomic DNA from whole blood samples was extracted by using salting out protocol. This method uses lysis buffer that contains detergent and salts and creates a hypertonic condition resulting in lysis of cells. The DNA concentration was measured by the spectrophotometer. The working solutions were obtained at a final concentration of 10 ng /μl and stored at -20 °C. All SNPs were performed using a StepOne Real-time PCR system (Applied Biosystems) and commercial kits TaqMan SNP Genotyping Assay™ 40X (Assay IDs: C__29809139_20, C__29086771_20, C__29086780_10, C___2149241_10, Applied Biosystems). PCR reactions were done according to the manufacturer's protocol with a final volume of reaction 15 μl per well. The program used considered an initial step of 10 minutes at 95 ° C, followed by 40 cycles at 95 ° C of 15 seconds each, and one minute at 60 ° C. Allelic Discrimination was determined by the Step One software applying the fluorescence probes. The Fluorescence values were detected in the FAM channel for the allele 1 and VIC channel for the allele 2. The dye used as the passive reference was ROX.

3.2.3 Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested for the control SNPs. Descriptive analysis using proportion and means ± standard deviation was computed for categorical and quantitative variables. Differences between groups were calculated using chi-squared and two-sample t-tests.

Association of IPMN with named SNPs was assessed by fitting unconditional multiple logistic regression models both to investigate departure from the multiplicative model and to identify the effect model best fitting the data. We started by modelling the relationship between IPMN and the genetic markers to try to underpin the transmission inheritance model. We fitted the regression model at a genotype level by assuming the following genetic models: not assuming any model, a dominant, a recessive and

multiplicative. A Likelihood Ratio Test was used to check the departure from a multiplicative model. Finally, ORs of IPMN and corresponding 95% CI according to analysed polymorphisms were derived from unconditional multiple logistic regression models using the multiplicative model, including terms for age and sex.

We also examined the possible confounding effect of smoking, alcohol, and cancer family history. However, models including these covariates yielded very similar results. Thus, given the small numbers in some strata, only the age- and sex-adjusted estimates were presented. Finally, Results were stratified according to any cancer 1st-degree family history.

3.3. The colonoscopic case-control study

Colonoscopic case-control study was conducted in order to assess whether patients with pancreatic IPMN have an increased propensity to develop colorectal adenomas.

3.3.1 Study design

A two centre prospective case-control study was conducted at the Digestive Endoscopy Unit of the Catholic University and the Digestive and Liver Disease Unit of the University Sapienza, Rome, Italy between January 2012 and December 2013. Cases were prevalent IPMN who underwent screening colonoscopy for the first time in their life. The criteria for the IPMN diagnosis have been previously described (78). The diagnosis of IPMN was considered as certain in the presence of either histological diagnosis obtained by EUS or surgical specimen, or cytologic diagnosis obtained by EUS. A highly probable diagnosis of IPMN was based on the presence of one or several main pancreatic duct and/or branch duct dilatation(s) and/or pancreatic cystic lesions communicating with pancreatic ducts at CT, S-MRCP, ERCP or EUS. Patients were excluded if they had a history of CRC, if they had undergone a previous colonoscopy independently on the presence or absence of CRC and/or colonic polyps, and if they had cystic lesions other than IPMN. Controls matched to each IPMN case (2:1) by gender and age (±5 years) were enrolled alongside among individuals who underwent their first colonoscopy for screening or for evaluation of non-specific abdominal symptoms at both institutions. Individuals who underwent colonoscopy because of a personal history

of CRC, of FAP or hereditary non-polyposis colorectal cancer (HNPCC), positivity to fecal occult blood test, iron deficiency anaemia and bright red blood per rectum were excluded. Both cases and controls were interviewed by trained physicians using a structured questionnaire and data on demographics, lifestyle habits (alcohol consumption, cigarette smoking), prior medical history and cancer family history were collected.

For IPMN cases data on IPMN characteristics, such as type of ductal involvement, focality, maximal dilation of the duct and presence of nodules or solid tissue inside the cyst cavity were collected. The type of duct involvement was determined by revision of clinical imaging studies and/or based on EUS evaluation, and classified as MD-IPMNs, BD-IPMNs or C-IPMNs.

3.3.2 Colonoscopies

Cases and controls underwent screening colonoscopy and the prevalence of colon polyps and/or colorectal cancer was determined. Polyethylene glycol lavage solution was used for colon preparation. Colonoscopies were conducted by experience endoscopists at the Digestive Endoscopy Unit of the Catholic University and the Digestive and Liver Disease Unit of the University Sapienza. Anatomical landmarks (Bauchini valve and appendix orifice) were recognized as proof that entire colon has been examined. Patients who were diagnosed with CRC were referred to surgical evaluation, while all detected polyps were removed when possible. Histopathologic examination of all removed polyps was performed and histologic type of the polyp and the degree of dysplasia were determined. All colonoscopies were performed under conscious sedation (midazolam and phentanyl e.v.) and patients were observed in recovery unit for 1 hour after the procedure. For those with positive colonoscopy findings, surveillance was suggested after proper treatment and histopathologic evaluation, according to current guidelines (81).

3.3.3 Statistical analysis

We conducted a descriptive analysis using relative frequencies and percentages to summarize the characteristics of the IPMN patients at the time of diagnosis. Chi-square

and Fisher exact tests were used to compare the distribution of categorical variables between patients with IPMNs and control subjects. Additionally, as we expect that the prevalence of colorectal polyps might differ between the compared groups based on the family history of CRC, we have planned a priori to stratify our data according to 1st-degree family history of CRC. Statistical analyses were performed using Stata software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

4. Results

4.1. The hospital-based multicentric study

Demographic of 390 IPMN patients included in the study are reported in Table 2.

Table 2. Demographics of 390 patients with intraductal papillary mucinous neoplasms (IPMN)s at the time of diagnosis

	IPMNs (*N*=390)	
Male	166	42.6%
Female	224	57.4%
Age (years)		
<50	36	9.2%
50-59	68	17.4%
60-69	139	35.6%
70-79	118	30.3%
>80	29	7.4%
Body-mass index (kg/m^2)	25	23-27

Data are median (IQR) for continuous variables or number of individuals (%) for discrete variables
(§) The percentage of missing value >5%

IPMN cases were predominantly female (57.4%). Most of the cases were of age 60-69 (35.6%) and 70-79 (30.3%), while 17.4%, 9.2% and 7.4% were of age 50-59, <50 and >80 respectively. Median body-max index of the patients included was 25 kg/m^2 (Table 2).

Table 3 reports the clinical characteristics of 390 IPMN patients included in the study. Three hundred and ten patients (79.5%) had BD-IPMN, 20 (5.1%) were diagnosed MD-IPMN, while 60 (15.4%) were having both branch and main duct involvement.

Table 3. Clinical features of 390 patients with intraductal papillary mucinous neoplasms (IPMN)s at the time of diagnosis

	IPMNs (*N*=390)	
Duct Involvement		
IPMN-BD	310	79.5%
IPMN-MD	20	5.1%
IPMN-mixed	60	15.4%
Focality		
Unifocal	142	36.5%
Multifocal	247	63.5%
Treatment		
Surgery	63	16.2%
No surgery	327	83.8%

Data are median (IQR) for continuous variables or number of individuals (%) for discrete variables
(§) The percentage of missing value >5%

Two hundred forty seven patients (63.5%) were diagnosed with more than one IPMN, while in 142 (36.5%) patients IPMN was unifocal. Only small portion of patients included (16.2%) underwent surgery in order to treat IPMN (Table 3).

The second primary malignancy was observed in 92 (23.6%) patients with IPMN; precisely 97 EPMs were diagnosed in 92 patients (Figure 6).

Figure 6. Occurrence of extrapancreatic malignancies (EPM)s among IPMN patients included in study

Patients with EPMs 23.6%

Patients without EPMs 76.4%
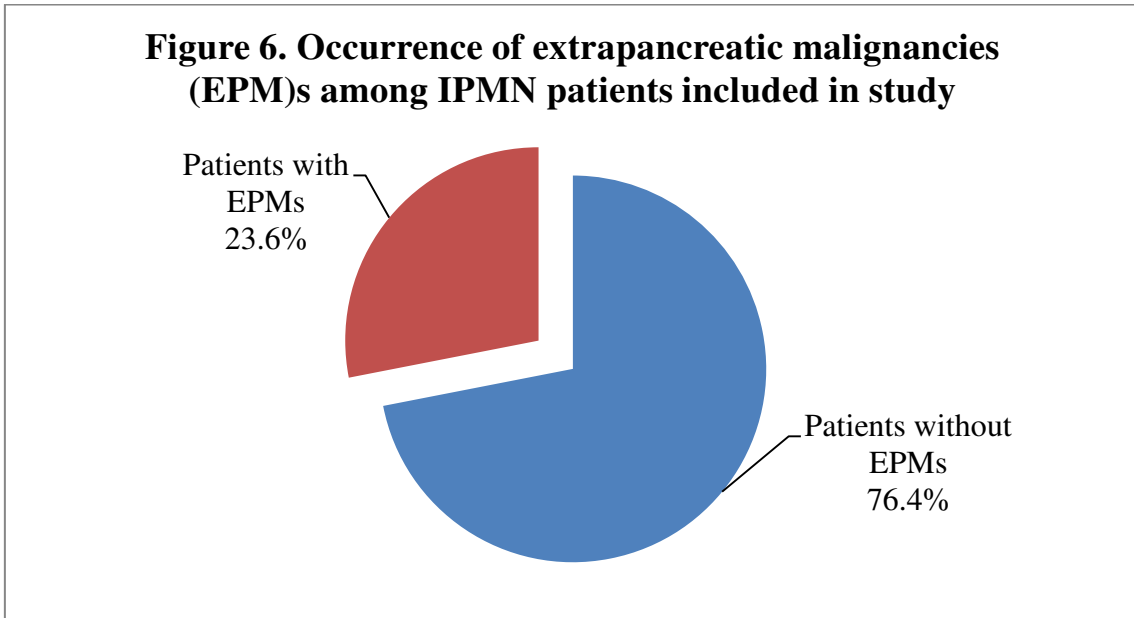
Seventy eight EPMs (80.4%) were previously diagnosed in 74 patients, 14 EPMs (14.4%) were diagnosed synchronous in 14 patients, and 5 (5.2%) were diagnosed metachronous in 4 patients (Figure 7).

Figure 7. Distribution of extrapancreatic malignancies (EPM)s according to time of diagnosis

Synchronous EPMs 15%

Metachronous EPMs 5%

Previous EPMs 80%
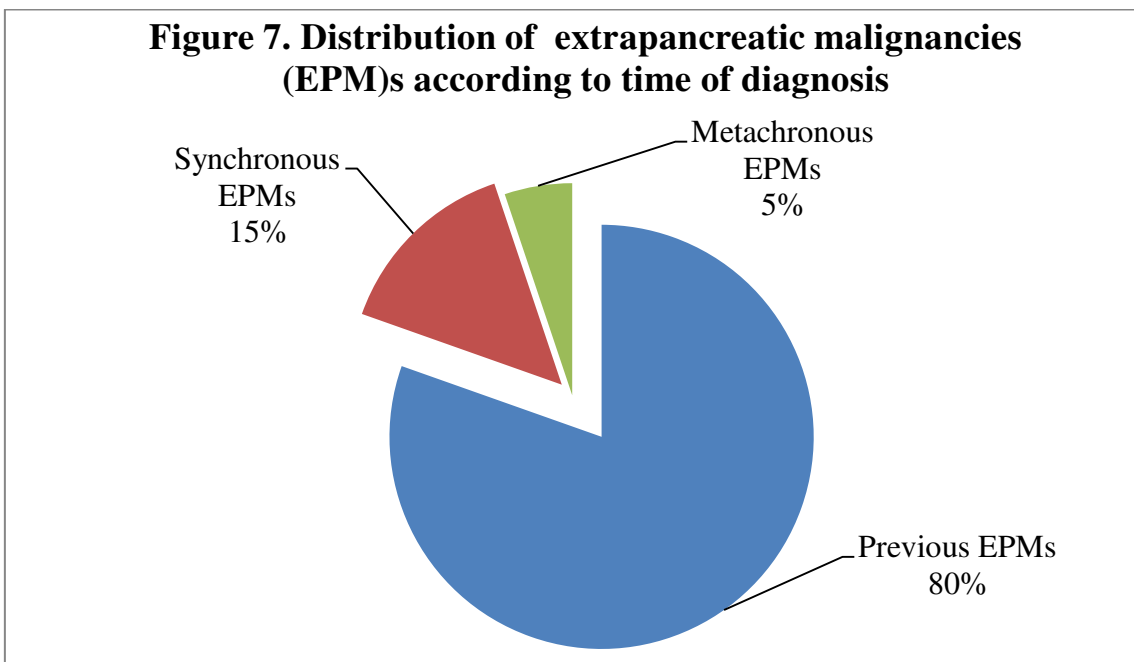
The most common tumors were breast cancer (15 cases, 15.5%), CRC (12 cases, 12.4%), renal cell carcinoma (9 cases, 9.3%), prostate carcinoma (8 cases, 8.2%), hematological cancers (7 cases, 7.2%) and thyroid carcinomas (5 cases, 5.2%) (Table 4).

Table 4. Extrapancreatic malignancies (EPMs) in patients with intraductal papillary mucinous neoplasms (IPMNs)

	Previous	Synchronous	Metachronous	Total
Breast (*)	12	3	0	15
Colorectal	7	5	0	12
Renal cell	8	1	0	9
Prostate	7	0	1	8
Hematological	6	0	1	7
Thyroid	5	0	0	5
Bladder	4	0	0	4
Hepatocellular	2	2	0	4
Lung	3	0	1	4
Melanoma	4	0	0	4
Non- melanoma skin	2	1	0	3
Ovary	3	0	0	3
Uterus	3	0	0	3
Gastrointestinal stromal	2	0	0	2
Meningiomas	2	0	0	2
Neuroendocrine gastrointestinal	1	0	1	2
Neuroendocrine lung	2	0	0	2
Acoustic neuroma	0	0	1	1
Ampullary intestinal-type	0	1	0	1
Gastric	1	0	0	1
Oesophageal	0	1	0	1
Oropharyngeal	1	0	0	1
Parotid adenocarcinoma	1	0	0	1
Thymoma	1	0	0	1
Ureter	1	0	0	1
Total	78	14	5	97

(*) Includes 1 male with breast cancer.
NOTE: Patients with EPMs are 92. Five patients have two cancers: breast + ovary, prostate + renal, prostate + hematological, uterus + breast, uterus + ovary.

Four cases of PDACs (1 previous and 3 synchronous) and 3 pancreatic neuroendocrine tumors (all of them synchronous) were also diagnosed.

The O/E ratios for previous and synchronous EPMs are reported in Table 5.

Table 5. Observed and expected number of extra-pancreatic malignancies (EPMs) in 390 patients with intraductal papillary mucinous neoplasms (IPMNs)

	Observed	Expected	O/E ratio[y]	CI 95%
Breast*	14	8.0	1.75	0.88-2.70
Colorectal	12	5.3	2.26	1.17-3.96
Renal cell	9	1.5	6.00	2.74-11.39
Prostate	7	4.0	1.75	0.64-3.28
Hematological	6	2.0	3.00	0.96-5.68
Thyroid	5	0.9	5.56	1.80-12.96

[y]O/E ratio=observed/expected ratio
*Calculated for females (1 male with breast cancer excluded)

A significantly increased occurrence of CRC (2.26, 95% CI: 1.17-3.96), renal cell carcinoma (5.33, 95% CI: 2.30-10.51) and thyroid carcinoma (5.66, 95% CI: 1.80-12.96) was observed in IPMN cases in comparison with Italian general population, while significance for hematological cancers (3.00, 95% CI: 0.96-5.68) was borderline (Table 5).

An increased risk for PDAC was also identified among IPMN patients (O/E=20, 95% CI: 5.41-51.21).

When comparing IPMN patients with and without EPMs according to demographics and lifestyle habits (Table 6), increasing age (p<0.05) and ever alcohol consumption (p<0.05) were observed as significant risk factors for EPM.

Table 6. Demographics and lifestyle habits of 390 intraductal papillary mucinous neoplasm (IPMN) cases with and without extrapancreatic malignancies (EPMs)

	Without EPMs (*N=298*)	**With EPMs** (*N=92*)	**p value**
Demographics			
Male Sex	127 (42.6%)	39 (42.4%)	0.97
Age	64.4 ±11.4	67.9 ±9.3	<0.05
BMI[γ]	25.1 ±3.9	25.1 ±3.9	0.88
Cigarette smoking			
Ever	153 (51.7%)	45 (51.1%)	0.9
<20 pack-years	54 (45 %)	9 (25%)	<0.05
≥20 pack-years	66 (55%)	27 (75%)	
Alcohol			
Ever	101 (34.5%)	41 (46.6%)	<0.05
≤20 units per week	60 (78.9%)	23 (79.3%)	0.36
>21 units per week	16 (21.1%)	6 (20.7%)	

[γ] BMI = Body-mass index (kg/m^2)

.

Although we did not observe that EPMs are significantly more common in ever smokers in compare to never smokers, those consuming ≥20 pack-years were significantly more represented among patients with EPMs (p<0.05) (Table 6).

When comparing IPMN patients with and without EPMs according to medical history and cancer family history (Table 7), 1st degree family history of PDAC (p<0.01), medical history of peptic ulcer (p<0.05) and 1st degree family history of gastric cancer (p<0.05) were observed to act protectively on the occurrence of EPMs.

Table 7. Characteristics of 390 intraductal papillary mucinous neoplasm (IPMN) cases with and without extrapancreatic malignancies (EPMs) by selected covariates.

	Without EPMs (*N*=298)	With EPMs (*N*=92)	p value
Clinical history			
History of chronic pancreatitis	10 (3.4%)	2 (2.2%)	0.74
History of diabetes	40 (13.4%)	13 (14.3%)	0.83
Recent-onset diabetes	4 (1.3%)	1 (1.1%)	1.00
History of peptic ulcer	26 (8.7%)	2 (2.2%)	<0.01
History of cholecystectomy	42 (14.5%)	10 (11.0%)	0.40
Cancer family history			
Any cancer (1st degree)	131 (44.3%)	50 (54.3%)	0.09
Any cancer (2nd degree)	46 (15.5%)	10 (10.9%)	0.27
PDAC* (1st degree)	21 (7.1%)	0 (0.0%)	<0.005
PDAC (2nd degree)	8 (2.7%)	5 (5.4%)	0.20
IPMN (1st degree)	2 (0.7%)	2 (2.2%)	0.23
Cancer sites (1st degree)			
Colorectal cancer	27 (9.1%)	15 (16.3%)	0.05
Gastric cancer	12 (4.0%)	9 (9.8%)	<0.05
Breast cancer	16 (5.4%)	6 (6.5%)	0.68
Lung cancer	21 (7.1%)	6 (6.5%)	0.86
Uterine cancer	9 (3.0%)	5 (5.4%)	0.33
Melanoma	3 (1.0%)	0 (0.0%)	1.00
Hepatocellular carcinoma	12 (4.0%)	3 (3.3%)	1.00
Drugs			
Use of aspirin	39 (13.1%)	10 (11.1%)	0.62
Use of statins	36 (12.1%)	11 (12.2%)	0.97
Use of insulin	13 (4.4%)	6 (6.6%)	0.41

*PDAC = pancreatic ductal adenocarcinoma

First degree family history of CRC was borderline significant risk factor for EPM (p=0.05) (Table 7). The occurrence of EPMs was more frequent among non-surgical IPMN patients (p<0.005)

4.2. The genetic-association study

The demographics of 117 IPMN cases and 231 controls included in the study are reported in Table 8.

Table 8. Demographics of 117 intraductal papillary mucinous neoplasm (IPMN) cases and 231 controls

	IPMN cases N (%)	Controls N (%)	
Age (years)	63.5 ± 11.8*	63.8 ± 12.5*	p=0.81
Gender			
Male	70 (59.8)	135 (58.4)	p=0.80
Female	47 (40.2)	96 (41.6)	

*mean±standard deviation (SD)

Cases were of mean age 63.5 ± 11.8, while controls were 63.8 ± 12.5 years old. Among cases 59.8% were males in compare to 58.4% in controls.

The cancer family history and lifestyle habits of 117 IPMN cases and 231 controls included in genetic association study are reported in Table 9.

Table 9. Distribution of 117 intraductal papillary mucinous neoplasm (IPMN) cases and 231 controls according to lifestyle habits and cancer family history

	IPMN cases N (%)	Controls N (%)	
Age (years)	63.5 ± 11.8*	63.8 ± 12.5*	p=0.81
Gender			
Male	70 (59.8)	135 (58.4)	p=0.80
Female	47 (40.2)	96 (41.6)	
Any cancer 1st degree family history[a]	71 (62.3)	65 (30.1)	p<0.001
CRC[γ] 1st degree family history[a]	15 (13.2)	16 (7.5)	p=0.10
Smoking[a]			
Never	47 (41.0)	138 (59.7)	
Ex-smoker	15 (12.9)	32 (13.9)	p=0.001
Current-smoker	8 (6.9)	15 (6.5)	
>20 pack/years	46 (39.3)	46 (20.0)	
Alcohol gr/day			
< 12 gr/day	51 (43.6)	147 (63.6)	
12-23 gr/day	13 (11.1)	47 (20.4)	p<0.001
> 23 gr/day	53 (45.3)	37 (16.2)	

[γ]CRC=colorectal cancer
[a]The sum does not add up to the total because of missing values.
*mean±standard deviation (SD)

Cases were more likely to report any cancer 1st degree family history than controls (p<0.001). Among cases was significantly more heavy smokers (p=0.001) and heavy drinkers (p<0.001) (Table 9).

Table 10 reports the distribution of cases and controls, ORs and 95% CIs for IPMN according to the selected SNPs (Table 10).

Table 10. Distribution of cases and controls, according to studied single-nucleotide polymorphisms (SNP)s and their association with intraductal papillary mucinous neoplasms (IPMN)s

	IPMN cases (N=117)		Controls (N=231)		p-value[a]	OR[*](95% CI)[ᵛ]
	N	%	N	%		
rs10505477					0.29	
wt[i]/wt	26	23.01	60	30.45		1
wt/mt[†]	61	53.98	113	51.36		1.30 (0.93-1.82)
mt/mt	26	23.01	40	18.18		1.69 (0.86-3.31)
rs6983267					0.28	
wt/wt	21	18.58	57	25.91		1
wt/mt	68	60.18	115	52.27		1.16 (0.82-1.62)
mt/mt	24	21.24	48	21.82		1.35 (0.67-2.62)
rs7014346					0.57	
wt/wt	37	32.74	85	38.46		1
wt/mt	61	53.98	111	50.23		1.20 (0.85-1.70)
mt/mt	15	13.27	25	11.31		1.44 (0.72-2.89)
rs6993464					0.57	
wt/wt	30	26.55	71	32.13		1
wt/mt	61	53.98	111	50.23		1.09 (0.88-1.37)
mt/mt	22	19.47	39	17.65		1.19 (0.77-1.88)

[a]p-value of the chi square test

[*]OR=odds ratio; [ᵛ]95% CI=95% confidence interval.

[i]wt=wild-type allele.

[†]mt=variant-type allele

No significant association was observed between the selected SNPs and IPMN (Table 10).

4.3. The colonoscopic case-control study

One hundred and twenty-two patients with IPMN and 244 matched controls were prospectively enrolled in the study. Table 11 reports demographic characteristics of the cases included in the study.

Table 11. Demographics of the 122 patients with intraductal papillary mucinous neoplasms (IPMN)s of the pancreas at the time of diagnosis

	Number	%
Gender		
Male	47	38.5%
Female	75	61.5%
Age		
<50	15	12.3%
50-59	22	18.0%
60-69	43	35.2%
≥70	42	34.4%

IPMN were predominantly female (61.5%), with an age greater than 60 years in 69.6% of the cases (Table 11).

Table 12 reports the clinical features of the IPMN cases included in the study (Table 12)

Table 12. Clinical features of the 122 patients with intraductal papillary mucinous neoplasms (IPMN)s of the pancreas at the time of diagnosis

	Number	%
Duct Involvement		
BD-IPMN[*]	107	87.7%
MD-IPMN[i]	7	5.7%
C-IPMN[γ]	8	6.6%
Focality		
Unifocal	40	32.8%
Multifocal	82	67.2%
Branch duct maximum dilatation (mm)[§]	16.69	12.0-21.0
Wirsung maximal dilatation (mm)[§]	9.0	5.0-10
Nodules		
No	99	81.1%
Yes	23	18.9%
Surgery		
No	106	86.9%
Yes	16	13.1%
Endosonography		
No	27	22.1%
Yes	95	77.9%

[*]BD-IPMN=branch duct
[i]MD-IPMN=main duct
[γ]C-IPMN=combined
[§]Median and interquartile range

The large majority of the cases were BD-IPMN (87.7%) multifocal (67.2%) and without evidence of intra-lesional nodules (81.1%). The mean diameter of the largest lesions was 16.7±7.9mm, while the mean dilatation of the main pancreatic duct in MD-IPMN was 9.0±5.26mm (Table 8). EUS±FNA was performed in 80% of the IPMNs and overall they were treated conservatively with clinical follow up in 86.9% of the cases (Table 12).

Table 13 reports demographics and lifestyle habits of IPMN cases and controls (Table 13).

Table 13. Demographics and lifestyle habits of 122 cases with intraductal papillary mucinous neoplasms (IPMN)s of the pancreas and 246 controls included in study

	Cases (n=122)		Controls (n=246)		p value
Male sex	47	38.5%	94	38.2%	0.95
Age	63.4	±11.3	62.3	±10.9	0.25
Cigarette smoking					
Ever	64	53.8%	115	46.7%	0.21
≥20 pack-years	33	28.2%	48	19.5%	0.06
Alcohol					
Ever	53	44.5%	75	30.5%	<0.01
≥21 drinks per week	10	9.0%	7	2.9%	<0.05

Data are mean (SD) for continuous variables or number of individuals (%) for discrete variables

Cases were of mean age 63.4 ± 11.3, while controls were 62.3 ± 12.9 years old. Among cases 38.5% were males in compare to 38.2% in controls. There was no difference in age and gender structure of cases and controls. Cases were significantly more likely to be ever drinkers (p<0.01) or to drink ≥21 drinks per week (p<0.05) (Table 13).

Table 14 reports previous medical history and cancer family history of IPMN cases and controls (Table 14).

Table 14. Characteristics of 122 patients with intraductal papillary mucinous neoplasms (IPMN)s of the pancreas and 246 controls by selected variables of medical history and cancer family history

	Cases (n=122)		Controls (n=246)		p value
Medical history					
History of chronic pancreatitis	8	6.7%	0	0.0%	<0.001
History of diabetes	23	19.3%	18	7.3%	<0.001
History of insulin-dependent diabetes	6	5.0%	0	0.0%	<0.001
History of noninsulin-dependent diabetes	17	14.3%	16	6.7%	<0.05
History of peptic ulcer	4	3.3%	3	1.2%	0.23
History of cholecystectomy	20	16.7%	17	6.9%	<0.01
Cancer family history					
Any cancer (1st degree)	70	57.9%	134	57.8%	0.99
Any cancer (2nd degree)	22	18.2%	24	10.3%	<0.05
PDAC[*] (1st degree)	12	9.9%	13	5.3%	0.10
PDAC (2nd degree)	4	3.3%	0	0.0%	<0.05
Common sites, first-degree family history					
Colorectal cancer	17	14.1%	93	37.8%	<0.001
Gastric cancer	9	7.4%	12	4.9%	0.32
Breast cancer	6	5.0%	21	8.5%	0.22
Lung cancer	12	9.9%	18	7.3%	0.39
Uterine cancer	3	2.5%	4	1.6%	0.69
Melanoma	3	2.5%	3	1.2%	0.40
Hepatocellular carcinoma	6	5.0%	5	2.0%	0.19
Drugs					
Use of aspirin	16	13.2%	34	14.3%	0.78
Use of statins	20	16.5%	49	20.6%	0.36
Use of insulin	7	5.8%	0	0.0%	<0.001

Data are mean (SD) for continuous variables or number of individuals (%) for discrete variables
[*]PDAC=pancreatic ductal adenocarcinoma

Cases were more frequently associated with history of chronic pancreatitis (p<0.001), diabetes mellitus (p<0.001), both insulin-dependent (p<0.001) and non-insulin dependent (p<0.05), as well as previous history of cholecystectomy (p<0.01) (Table 14).

In regards to cancer family history, IPMN patients were significantly more likely to have 2nd-degree family history for any cancer (p<0.05), and 2nd-degree family history for PDAC (p<0.05). On the other hand, controls were significantly more likely to have a 1st-degree relative with history of CRC (p<0.001) (Table 14).

In all of the cases and controls entire colon was examined. Table 15 reports polyp findings among cases and controls according to colonoscopy.

Table 15. Polyp findings among 122 patients with intraductal papillary mucinous neoplasms (IPMN)s of the pancreas and 246 controls according to colonoscopy

	Cases (n=122)		Controls (n=246)		p value
Colon polyps	52	42.6%	79	32.1%	<0.05
Adenomatous polyps	29	23.8%	57	23.2%	0.9
Multiple adenomatous polyps	11	9.0%	20	8.1%	0.77
Low grade dysplasia	24	19.7%	48	19.5%	0.98
High grade dysplasia	6	4.9%	11	4.5%	0.85

Data are mean (SD) for continuous variables or number of individuals (%) for discrete variables

Colorectal polyps were found in 52 IPMNs (42.6%) and in 79 controls (32.1%) (p<0.05) (Table 15). Mean polyp diameter was 6.1±5.28mm. In 29 cases (23.8%) and 57 controls (23.2%) histological examination disclosed adenomatous polyps (p=0.90), which were multiple in 11 cases (9%) and 20 controls (8.1%) (p=0.77) (Table 15). There was no difference between the groups in regard to presence of polyps with low-grade dysplasia (19.7% vs. 19.8%, p=0.98) or high-grade dysplasia (4.9% vs. 4.5%, p=0.85) (Table 15).

Three cases of CRC were detected, 2 among IPMNs (1.6%) and 1 among controls (0.4%), but the frequency was too small to make any comparison between the groups.

As we expected that 1st-degree family history of CRC might affect the prevalence of colorectal polyps, Table 16 reports the data stratified according to this covariate (Table 16).

Table 16. Prevalence of colon polyps in intraductal papillary mucinous neoplasms (IPMN)s of the pancreas cases and controls according to family history of colorectal cancer (CRC)

1st-degree family history of CRC	Cases (n=104)		Controls (n=153)		p-value
Colon polyps	45	43.4%	48	31.4%	0.051
Adenomatous polyps	26	25.0%	36	23.5%	0.79
Multiple adenomatous polyps	10	9.6%	13	8.5%	0.76
Low grade dysplasia	21	20.2%	30	19.6%	0.90
High grade dysplasia	8	7.7%	8	5.2%	0.88
No 1st-degree family history of CRC	**Cases (n=17)**		**Controls (n=93)**		**p-value**
Colon polyps	6	35.3%	31	33.3%	0.85
Adenomatous polyps	3	17.7%	21	22.6%	0.65
Multiple adenomatous polyps	1	5.9%	7	7.5%	0.81
Low grade dysplasia	3	17.6%	18	19.3%	0.85
High grade dysplasia	1	5.9%	3	3.2%	0.59

Data are number of individuals (%)

No significant difference in the prevalence of colorectal polyps or adenomatous polyps with low-grade or high-grade dysplasia was observed between IPMN cases and controls without 1st degree family history of CRC (Table 16).

However when restricting the analysis on cases and control with 1st degree family history of CRC only, we observed a borderline significantly higher prevalence of colorectal polyps among IPMN cases (43.3% vs. 31.4%, p=0.051) (Table 16). Nevertheless, when taking into consideration only adenomatous polyps, no significant difference between IPMN cases and controls with 1st degree family history of CRC was observed (Table 16).

5. Discussion

Our study is the first multicentric study of IPMNs conducted in Europe. Results show that 23.6% of IPMNs experience EPMs. The rate of prevalent EPMs was higher than expected from the general reference population, especially for CRC, renal cell and thyroid cancers. Additionally, we reported that increased age, alcohol consumption and 1st-degree family history of gastric cancer are associated with the occurrence of any EPMs among IPMN patients, while 1st-degree family history of CRC was borderline significant.

Number of authors so far addressed the association of IPMN with EPMs (Table 1). First study was conducted in 1999 by Sugiyama et al. (50). They conducted a study on 42 patients who underwent surgery because of the IPMN, focusing on the incidence and characteristics of nonpancreatic neoplasms. Fifteen (32%) among them were reported with EPMs. Most frequently reported EPMs were CRC (11.9%) and gastric cancer (9.5%). Only risk factor significantly associated with occurrence of EPMs was age.

Followed the study by Osanai et al. (51), conducted on 148 IPMN patients, including both those treated surgically and those not. Thirty-five patients (23.6%) experienced EPMs. Of those 11 were diagnosed with CRC (7.4%), 8 with gastric cancer (5.4%), and 5 with lung cancer (3.4%). Similar study was conducted by Kamisawa et al. on 79 cases of IPMN diagnosed by detection of mucous in the pancreatic duct during ERCP (52). Forty EPMs occurred in 28 patients (35.4%). Major associated EPMs were gastric cancer (15.2%), CRC, (8.9%), esophageal cancer (5.1%) and lung cancer (5.1%). Development of EPMs was significantly related to age. Choi et al. (53) set up a study in order to estimate incidence and clinicopathological features of extrapancreatic neoplasms in patients with IPMN. They enrolled 61 patients who underwent surgical resection for IPMN. Twenty-four (39%) among them developed 26 extrapancreatic neoplasms, and 18 (30%) had EPMs. Gastric adenocarcinoma (33%) and CRC (17%) were reported to be the most common EPMs.

Another study from Japan was conducted by Eguchi et al. (54). They screened the records of 69 surgically treated IPMN patients in order to assess the risk factors for preoperative or postoperative EPMs. This also was the first study trying to calculate the rate of increase of EPMs in IPMN patients, compared with the normal population. The O/E ratios were calculated by using the Osaka Cancer Registry. The preoperative EPMs

were diagnosed in 38% of patients. Most frequently diagnosed was CRC (11.6%), followed by gastric cancer (5.8%). The O/E ratio of preoperative EPMs was significantly high in IMPN patients (2.41; 95% CI, 1.51-3.64). Furthermore O/E ratio for preoperative CRC was also significant (5.37; 95% CI, 2.31-10.58). Logistic regression analysis showed IPMN and age to be independent risk factors for preoperative CRC development. During the postoperative follow-up, 10 IPMN patients (15%) developed EPMs and 3 died from it.

After these initial studies conducted in Asian population came the study by Riall et al. (55) conducted in the USA. They performed a population-based observational cohort study in order to determine the incidence and site of additional EPMs in patients with invasive IPMN. Noninvasive IPMNs were not included in the analysis. In order to obtain the data needed, they used Surveillance, Epidemiology, and End Results (SEER) tumor registry database. The registry is sponsored by the National Cancer Institute and contains over 3 million cancer cases, with 170.000 new cases added annually. Authors identified 992 case of invasive IPMN reported in the period 1973-2001. Among these, 100 patients (10.1%) developed EPMs. The most frequent were CRC (2.5%), breast cancer (1.5%) and prostate cancer (1.4%). In addition, authors calculated the O/E ratio based on the 2006 Cancer Statistics and the 2005 estimated USA population for the USA Census Bureau. The observed rate of CRC was 1.66 times the rate expected in the general population. Rates of breast and lung cancer were also increased, with O/E ratios of 1.13 and 1.22, respectively.

First study from European population was conducted in France by Baumgaertner et al (56). Authors set up a case–control study comparing 178 patients with resected IPMN with 356 age- and gender-matched controls in order to assess the association of IPMN with EPMs. Ninety-one of IPMN patients have been verified with hyperplasia/low-grade dysplasia and 87 with high-grade dysplasia/invasive cancer. EPMs were found in 30 of 178 (16.8%) patients with IPMN, 70% of which preceding IPMN. The most frequent EPMs in IPMN patients were breast cancer (5.4%), prostate cancer (2.2%) and CRC (1.7%). The grade of dysplasia in IPMN was not associated with EPM occurrence.

What followed were another three studies from Asia, one from Japan (57) and two from South Korea (58, 59). Ishida et al. (57) conducted a study on 61 patient who underwent surgery because of IPMN in Tohoku University Hospital between 1988 and 2006. Thirty-six of these were diagnosed with intraductal papillary-mucinous carcinomas

including 6 with invasive carcinomas. Synchronous and metachronous EPMs were observed in 15 out of the 61 patient (24.6%). Among them gastric cancer was observed in 10% of IPMNs and CRC in 8%. None of the features, including sex, age, smoking, family history, macroscopic types (main duct type or branch duct type) and histological types (gastric, intestinal, pancreatobiliary or oncocytic) was associated with EPMs. However, EPMs were more frequent among malignant than benign IPMNs. Oh et al. (59) reported from the small series of 37 IPMN patients confirmed either by surgical resection or typical findings of EUS and CT imaging. Ten (27%) IPMNs were associated with EPMs. Gastric cancer (3 patients, 8.1%) and CRC (3 patients, 8.1%) were the most common neoplasms. Finally the biggest study in Asian population so far was conducted by Yoon et al. (58). They aimed to assess the prevalence and associated factors of EPMs in IPMN patients and to compare it with those of non-IPMN pancreatic cystic neoplasm patients. Number of 210 IPMNs and 175 patients with other cystic neoplasm of the pancreas was included in the study. The prevalence of EPM was 33.8% for IPMNs and was significantly higher than in patients with other cystic neoplasms of pancreas. Most frequent among EPMs was gastric cancer (13.8%), followed by CRC (7.6%) and bile duct cancer (3.8%). Age was significantly associated with occurrence of EPMs in IPMN patients, while malignant IPMN showed a borderline inverse association.

Another interesting study came from the Mayo Clinic. Reid-Lombardo et al. (60) identified all patients diagnosed with IPMN at the named institution from 1994 to 2006 in order to estimate the frequency of EPMs and compare it to one in patients with ductal pancreatic cancer and a general referral population. Four hundred seventy one patients with IPMN were enrolled. Among them 40.8% were diagnosed with 192 EPMs before or coincident with IPMN. The most common EPMs were non-melanoma skin cancer (7.5%), breast cancer (5.1%), prostate cancer (5.1%), CRC (4.0%), and carcinoid neoplasms (1.3%). IPMN patients were at significantly higher risk to harbor hepatobiliar (OR: 3.0, 95% CI: 1.1–8.1), esophageal (OR: 5.5, 95% CI: 1.8–16.5), and gastrointestinal stromal tumors (OR: 3.8, 95% CI: 1.0–14.1) in compare to general referral population. Furthermore, occurrence of colonic polyps was registered in high number of IPMN cases (114, 24%). As IPMN patients were at significantly higher risk to develop colonic polyps (OR: 1.9, 95% CI: 1.4–2.4) authors suggested screening colonoscopy to be considered in all of them.

Lubecky et al. (61) reported the results originating from series of 82 patients diagnosed in single center in Israel. They intended to evaluate the association of IPMN with EPMs, but also to assess the influence of cancer family history and germline BRCA1 and BRCA2 mutations to the named association. They reported EPMs in 19.6% of IPMN patients. Most frequent were CRC (6.1%), breast (3.7%) and prostate cancer (3.7%). There was an increased rate of cancer in families of IPMN patients; however this difference did not reach statistical significance. Nevertheless, a significantly higher rate of CRC in families of IPMN patients who had EPMs was observed. Based on these findings authors suggested a genetic component in the pathogenesis of IPMN. Furthermore, they suggested that possible genetic changes include BRCA2, as BRCA2 mutations were found in 25% of IPMN patients with a family history of pancreatic cancer.

Only study present in the moment of initiation of our study that took into consideration only incident EPMs was study by Kawakubo et al. (62). They conducted a study on 642 Japanese IPMN patients in order to estimate the frequency of incident EPMs during the follow-up of 4.8 years on average. The incidence of the observed EPMs was compared with the expected incidence of the age- and gender-matched general Japanese population in order to calculate standardized incidence ratio (SIR). Forty EPMs developed in 39 patients (6.1%) during follow-up. The most common malignancies were hepatocellular (1.1%), colorectal (0.9%), gastric (0.9%), lung (0.8%) and prostate cancers (0.6%). They reported SIRs of 2.17 (95% CI 0.87-4.47) for hepatocellular cancer, 1.02 (95% CI 0.37-2.21) for CRC, 0.76 (95% CI 0.28-1.66) for gastric cancer, 0.75 (95% CI 0.24-1.76) for lung cancer and 1.00 (95% CI 0.71-1.29) for prostate cancer.

As can be seen, most of the studies available in the moment of initiation of our study were mainly in Asian populations (50-54, 57-59) (Table 1). Moreover most of the studies, included a small number of patients (<100) (50, 52-54, 57, 59), and many suffered of recruitment bias as IPMN patients were all surgical, thus including mainly malignant IPMNs (50, 53, 54, 57). These studies reported that the frequency of prevalent or incident EPMs was around 24.6%-38% (Table 1) (50, 53, 54, 57). Additional studies including surgical IPMNs and not, confirmed the higher frequencies of EPMs among IPMNs (52, 58, 59), with the largest study in Asian population (58) reporting a prevalence of EPMs of 33.8%. One study from US (60) reported a

prevalence of EPMs of 40.8% (60), while the only study conducted in Europe in the moment of initiation of our study reported an EPM occurrence of 16.8% (56). Unfortunately, most of these studies do not distinguish between prevalent and incident EPMs, so that results are hard to compare.

Our results are consistent with what was previously published, as we reported that 23.6% of IPMN patients experienced prevalent EPMs. Furthermore we showed significantly increased O/Es for CRC, renal cell carcinoma, thyroid and hematological cancers. Some authors before also tried to quantify the risk of EPMs in IPMN patients, comparing it to risk in control group (60) or general referral population (54, 55, 62). Eguchi et al. (54) showed a significantly increased O/E in the IPMN patients, especially for CRC. Rial et al. (55) reported increased risk for hepatobiliar, esophageal and gastrointestinal stromal tumors, and borderline significant risk for carcinoid and urinary tract tumors. Kawakubo et al. (62) performed long-term follow up of the IPMN patients and did not find any increased SIR of EPMs. Although in our study we diagnosed 5 metachronous cancers, our follow-up was too short to calculate SIRs. The follow-up is, however, undergoing so that additional reports on the incident of EPMs in our cohort are expected.

Two new studies on the subject were published recently by group of Marchegiani et al. (82, 83). They have put an accent on the incident EPMs in IPMN patients in order to calculate SIRs. Initially they conducted a study on 456 IPMN patients diagnosed at single center in Verona, Italy who were followed according to guidelines at a median follow-up of 56 months (82). The incidence of EPMs was calculated only in patients who were free of them at the time of IPMN diagnosis. Data were compared with Italian cancer statistics in order to calculate SIRs. Thirty EPMs developed during the follow-up with cumulative incidence of 6.6%. Authors reported SIR of 1.35 (95% CI, 0.91–1.93). After stratification according to gender SIR was 1.40 (95% CI, 0.72–2.45) in males and 1.37 (95% CI, 0.81–2.16) in females. When the analysis was stratified by single tumors, authors observed a significantly increased incidence of melanoma in females (SIR 5.56; 95% CI, 1.12–16.26).

Subsequently, same group published multicentric study on 816 patients with IPMNs evaluated from 2000 through 2013 at 4 academic institutions in Europe for development of extrapancreatic neoplasms (83). The incidence of extrapancreatic neoplasms was compared with sex-specific, age-adjusted European cancer statistics in order to calculate

SIR. Among the 816 patients included in the incidence analysis, 50 developed an extrapancreatic neoplasm after a median time of 46 months from study enrollment. The authors reported SIR of 1.48 (95% CI, 0.94-2.22) in males and of 1.39 (95% CI 0.90-2.05) in females.

Having in mind results of these two studies (82, 83) Marchegiani study group concluded that patients with IPMN do not have a significantly higher incidence of extrapancreatic neoplasms than the general population. However, although these two studies are so far only prospective studies in European population reporting SIRs for EPMs in IPMN patients, their results should be interpreted with caution. Above all, both studies actually reported borderline risks for EPMs among IPMNs, as single-centre study from Verona reported SIR of 1.35 (95% CI, 0.91–1.93) for both genders (82) while multicentric study reported SIR of 1.48 (95% CI, 0.94-2.22) in males and of 1.39 (95% CI 0.90-2.05) in females (83). Furthermore in the first study authors reported significantly increased incidence of melanoma in females (82). If we have in mind that the follow-up in these studies was relatively short it could be a case that after a longer follow-up in the same cohort these result would not be borderline any more, but significant. Therefore, in order to estimate the association between IPMN and incident EPMs, longer follow-up is needed. Larger multicentric European studies with longer follow-up could give an answer to this question in the future.

Several studies showed that patients with IPMN have an increased risk of EPMs compared to those with PDAC (50, 53, 54, 60, 61), even though this is not true for malignant IPMN (55). In fact several reports also suggest that an inverse association exists between IPMN malignant potential and EPM frequency (57, 58). This observation was confirmed also in our study, as EPMs were borderline more frequent in non-surgical IPMNs. IPMN is considered to be a precursor lesion for PDAC (84), and patients with IPMNs have been reported to be at the increased risk to develop coexistent PDAC (48). Our study showed an increased O/E ratio for PDAC in IPMNs, speaking in favor of the "precursor lesion" theory. Unexpectedly, however, we observed a protective effect of 1st-degree family history of PDAC to occurrence of EPMs in IPMN patients. As patients with 1st-degree family history of PDAC are reported to more likely develop PDAC (85), it is possible that the early onset of PDAC with aggressive progression could lead to insufficient time left for EPMs to occur.

Our study confirmed the role of increased age as risk factor for EPMs in IPMN (50, 52, 55, 58), which is expected also in view of the well-known increased risk of cancer overall in older population (86). We identified 1st-degree family history of gastric cancer as a risk factor for EPMs, while 1st-degree family history of CRC was borderline. A recent study in Japan reported that individuals with 1st-degree family history of gastric cancer are at the increased risk to develop CRC (87), and this supports our finding as CRC is the second most common form of EPMs. Still, this result needs to be confirmed in a larger study, as it might be a peculiar finding only among the IPMN patients. First degree family history of CRC was reported as a risk factor for EPMs by Lubezky et al. (61), while another report implicated that IPMNs are associated with FAP syndrome (64). We were unable to find a significant association, though our borderline finding result is in line also with the knowledge that a large proportion of the EPMs are CRC, for which a positive family history is acknowledged (88).

Concerning cigarette smoking habits, our study confirms that smoke is not associated with EPMs (53, 56), however we report that smokers who consumed ≥20 pack-years are more likely to have EPMs compared to <20 pack-years. Although alcohol has been previously investigated as a possible risk factor for EPMs among IPMNs (53, 56), our study is the first reporting a significant association. As alcohol has been reported as one of major contributors to cancer risk (89), it is conceivable that it can influence the occurrence of EPMs. For the first time we report a protective effect of peptic ulcer history towards EPMs in IPMNs, which need to be further investigated within studies that collected this information.

Main limitation of this study is that we took into account only prevalent (synchronous and metachronous) and not incident EPMs. The follow-up of the patients was too short to refer also to incident EPMs and calculate SIR, so we limited our report only to O/E ratio generated from observed prevalent EPMs. However the follow-up is under way and it is expected that these results will be published in future. Diagnostic bias might be an issue, as EPMs might be overestimated in IPMN patients respect to the general population as these subjects are usually under increased medical observation. We feel, however, to exclude the presence of this bias as the vast majority of EPMs were diagnosed previous or concomitant with IPMN diagnosis, with IPMN actually diagnosed soon after the EPMs diagnosis.

The genetic association study we conducted did not find any of the named SNPs (rs6983267, rs6993464, rs7014346, rs10505477) in human chromosomal region 8q24 to be associated with IPMN.

Since IPMN was reported to be associated with an increased risk of developing EPMs (51-61), especially CRC (50-54, 58, 59, 61), it has been hypothesized that common genetic background is responsible for the observed association. Lubecky et al. (61) found an increased rate of cancer in families of IPMN patients, specifically CRC. Based on these findings they suggested a genetic component in the pathogenesis of IPMN. Furthermore authors hypothesized that possible genetic changes include BRCA2. Nevertheless, none study so far ever attempted to test the hypothesis of the genetic background of the association between IPMN and EPMs.

SNPs in human chromosomal region 8q24 are reported to be associated with cancer in general and, particularly, CRC (70). The majority of these associations lie at approximately 128 Mb on chromosome 8. Some of the SNPs have been shown to interact with the proto-oncogene MYC (68). MYC is an important protooncogene, over-expressed in numerous tumors, including CRC. The region contains several other genes which could be functionally related to cancer development, including NOV and ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2). NOV encodes a regulatory protein from the CCN family that has been associated with cancer development (69). ENPP2 encodes a phospholipase which stimulates tumor cell motility and proliferation (90). In addition it has been reported (91) that both NOV and ENPP2 are indirectly regulated by the 8q24 proto-oncogene MYC, via p53 (for NOV) (92, 93) and ESR2 (for ENPP2) (94, 95), what makes their potential involvement in a pathway for cancer susceptibility plausible. In light of the named facts, it can be supposed that this region contains a locus for general cancer susceptibility.

Recent studies have identified and confirmed associations of several SNPs within the region with CRC (66, 96, 97), breast (98, 99) and prostate cancer (100-103). Additional associations have been found for kidney, thyroid, and larynx cancer (104), as well as cancer of the upper aerodigestive tract (105). Three regions of 8q24 in section of 430Mb were initially found to be associated with prostate cancer, while two additional regions have been identified recently (106, 107). As for CRC, statistically significant associations are reported in an approximately 60 kb region of high linkage disequilibrium between chromosomal location 128.475 and 128.545 Mb (108).

In light of previously mentioned facts we identified 8q24 region as a good starting point in an initial research in order to enlighten genetic background of an association between IPMN and EPMs. Nevertheless, financial limitations did not make possible to investigate all the SNPs in the region, thus we needed to choose some of them. We judged that SNPs rs6983267, rs6993464, rs7014346, rs10505477 are most likely to be present in IPMN patients.

The SNP within 8q24 chromosome that so far showed highest significance for carcinogenesis and CRC in particular is rs6983267. There were several reports providing insights into the functional role of the rs6983267 (68, 109). Tuupanen et al. (109) reported that the risk allele G of rs6983267 shows copy number increase during CRC development. Furthermore, they showed that the SNP is located in a transcriptional enhancer and that the G allele has increased affinity for binding transcription factor 4 (TCF4) (109). The TCF4 is important in activating the transcription of Wnt target genes. Finally, they showed that the rs6983267 region physically interacts with the MYC promoter region (109). Based on these findings it can be concluded that the biological mechanisms associating the rs6983267 SNP to risk of CRC includes an impact on Wnt signalling and MYC expression (68, 109).

Number of studies so far addressed the impact of rs698267 to CRC risk (67, 70, 97, 103, 104, 110-117). All of them reported significant association of rs698267 with CRC. Hutter et al. (108) confirmed these findings by reporting modest but significant association (OR = 1.10; 95% CI: 1.01-1.20). Furthermore they conducted a meta-analysis including all the trials available in the moment, and the association was confirmed one more time as they reported OR of 1.21 (95% CI: 1.18-1.24). Newer meta-analyses by Brisbin et al. (118) confirmed these findings. Howevers they reported rs698267 to be associated not only to CRC but also to prostate cancer.

Aside from association of rs6993464 with CRC and prostate cancer, Brisbin et al. (118) also reported a novel SNP rs6993464, to be associated with cancer risk (p = 1.25E-07). The T allele of the SNP was associated with cancer risk in breast and pancreatic cancer (118). This SNP lies in the region between NOV and ENPP2. Several of the significant SNPs in this region are identified as expression quantitative trait loci (eQTLs) for genes throughout the genome being associated with various types of cancer (119). Among them rs6993464 has been shown to be an eQTL for POLR2F, a gene on chromosome 22 which is up-regulated in CRC (120).

The SNP rs7014346 on chromosome 8q24, is located on the POU class 5 homeobox 1 pseudogene 1 gene (POU5F1P1) (121). It has been suggested that that deregulated expression of POU genes in breast cancer cells could repress the expression of a tumor suppressor and activate the expression of an oncogenic growth factor (122). This SNP has been associated with increased risk to CRC (65, 108, 116)

The SNP rs10505477 is located in the intron of Cancer Susceptibility Candidate 8 (CASC8), a long non-coding RNA (lncRNA), which overlaps POU5F1B gene (123). It has been hypothesized that rs10505477 may disrupt the key regulatory region of CASC8, resulting in its miss-expression (123). In Miss-expressed CASC8 may modulate the recruitment of general transcription factors on the promoter of its cognate gene, POU5F1B, which was found to be a putative cancer susceptibility gene (124). By altering the fine turning interactions between the CASC8 and POU5F1B, the rs10505477 could be influencing cancer susceptibility (123). The rs10505477 has so far been associated with risk of CRC and breast cancer (66, 97, 125-127). Furthermore, Ma et al. (123) reported rs10505477 to be associated with the significantly lower survival rate in gastric cancer patients.

These findings gave a strong rationale to our decision to select the named SNPs to be tested for the association with IPMN. However, the results of our study show that none of the selected SNPs from 8q24 regions is significantly associated with IPMN, even when stratified according to 1st-degree family history of cancer.

Nevertheless in interpreting the results should be taken into consideration that due to relatively small number of included IPMN cases our study had limited power to identify weak but significant associations. Larger studies to come are needed in order to confirm our findings. Although we bring negative results, this is the first study addressing the genetic background of an association between IPMN and EPMs. Our finding should help other researchers to point their research in right direction, in order to further enlighten this subject.

We conducted a case-control study in order to evaluate the prevalence of colorectal adenomas in prospectively enrolled Italian patients with IPMN undergoing first time screening colonoscopy. Compared to a matched control population of individuals who underwent colonoscopy for screening or for evaluation of non-specific abdominal symptoms, no increase prevalence of adenomatous polyps was found in IPMN patients.

This result did not show an increased propensity to develop colorectal adenomas among IPMN patients.

CRC has been the most frequent EPM consistently found in IPMN patients (50-54, 58, 59, 61). Eguchi et al. (54) reported that IPMN is a strong independent risk factor for preoperative CRC. They found that CRC occurred 5.37 times more frequently in IPMN patients than in the general population. Rial et al. (55) reported 1.66 times higher rate of CRC in patients with invasive IPMN as compared to USA general population. In multicentric hospital-based study, that is also part of this thesis, we reported that IPMN patients in Italy harbor CRC 2.26 times more frequently than expected. Furthermore, Lubezky et al. (61) reported that first degree family history of CRC is a risk factor for EPMs in IPMN patients. Another report implicated that IPMNs are associated with FAP syndrome (64).

The mechanism(s) for the association between IPMN and CRC has not been elucidated so far. One important point has been raised by a study of Reid-Lombardo et al. (60), who performed a large case-control retrospective study at the Mayo Clinic. They found adenomatous colorectal polyps to be present 2 and 1.4 times more frequently in patients with IPMN as compared to patients with pancreatic adenocarcinoma and to the general population, respectively. These findings prompted the authors to advocate screening colonoscopy for all patients with IPMN.

The adenoma-carcinoma sequence is a well-known carcinogenetic mechanism that is responsible for almost all sporadic CRCs (76). Colorectal adenomas are benign tumors that arise from the glandular epithelium and project themselves above the surrounding mucosa. They are characterized by dysplastic morphology and altered differentiation of the epithelial cells in the lesion (128). Individuals in whom colorectal adenomas are not removed are at the increased risk for CRC and colonoscopic polypectomy lowers that risk substantially (129). Residuals of adenomatous tissue are often observed in CRC specimens and individual foci of carcinoma can be detected in adenomatous polyps (130). Individuals affected by conditions that include increased propensity to harobour colorectal adenomas, such as FAP, hereditary nonpolyposis colorectal cancer or Lynch syndrome, almost inevitably develop CRCs by third to fifth decade of their life if their colon is not removed (131, 132). CRC develops from the progressive transformation of adenomatous polyps through a series of molecular events. The initial step is loss of adenomatous polyposis coli (APC), that leads to adenoma formation (133). What

follows are the mutations in the small GTPase KRAS, acquired by larger adenomas and early carcinoma, followed by loss of chromosome 18q with SMAD4, which is downstream of transforming growth factor-β (TGFβ), and mutations in TP53 in frank carcinoma (133).

Based on this well-known mechanism and the results of the study by Reid-Lombardo et al. (60), we performed a study to test the hypothesis that the risk of CRC development in patients with IPMN could be related to an increased propensity to harbor colorectal adenomas. Differently from the study by Reid-Lombardo et al. (60) that was based on a retrospective chart review with possible bias toward an increased prevalence of adenomatous polyps in cases because of an increased probability of undergoing diagnostic test including colonoscopy, we evaluated the prevalence of colorectal polyps among a large cohort of consecutively enrolled patients with IPMN undergoing first time colonoscopy. As compared with a matched control population also undergoing first time colonoscopy for screening purposes or to evaluate non-specific symptoms, we found a higher prevalence of colorectal polyps among IPMN patients. When considering adenomatous polyps only, however, this difference was no longer significant. Furthermore, these results were confirmed after stratifying according to the 1st-degree family history of CRC, which might affect the presence of colorectal adenomas. Therefore our study did not confirm the findings of Reid-Lombardo et al. (60). However, when interpreting our results should be kept in mind that Reid-Lombardo et al. (60) included more MD-IPMNs and C-IPMNs. It could be a case that patients harboring these more invasive IPMN forms are more prone to develop colorectal adenomas.

Overall, the results of our study suggest that factors other than an increased propensity of IPMN patients to harbor colorectal adenomas should be responsible for the increased occurrence of CRC in these patients. It could be hypothesized that the mechanism for an increased risk of CRC in IPMN might be an accelerated adenomas formation, as seen in patients with HNPCC.

We observed several risk factors associated with IPMN. History of diabetes, use of insulin and history of chronic pancreatitis has been previously reported to be associated with IPMN (77). We found an association between cholecystectomy and IPMN, which has never been reported before and the meaning of which appears of an unclear value.

We acknowledge that our study has some limitations. It included a relatively limited number of IPMN cases, and because of small number of CRCs detected, it was underpowered to detect difference in CRC occurrence as compared to matched controls. However, the study was designed to assess the prevalence of adenomas and not CRC among IPMN. Furthermore, significantly higher rate of chronic pancreatitis and heavy drinking in the IPMN group suggests misclassification indicating that some of the cystic lesions may be pseudocysts rather than IPMNs. However, this is the first study assessing the prevalence of colorectal adenomas in IPMN patients at first time colonoscopy. Although the results reported were against our prior hypothesis of higher prevalence of colorectal adenomas among IPMN than the control population, our study highlighted the need to further explore the issue of association between IPMN and CRC.

6. Conclusions

1. Patients with intraductal papillary mucinous neoplasms of pancreas are at higher risk of developing colorectal cancer than general population of Italy.

2. Patients with intraductal papillary mucinous neoplasms of pancreas are at higher risk of developing renal cell carcinoma than general population of Italy.

3. Patients with intraductal papillary mucinous neoplasms of pancreas are at higher risk of developing thyroid carcinoma than general population of Italy.

4. Further investigations in other European populations are expected in order to confirm these findings.

5. Systematic surveillance of intraductal papillary mucinous neoplasms cases for the colorectal, renal cell and thyroid carcinoma is advised.

6. Increased age, heavy cigarette smoking and alcohol consumption are significant risk factors for extrapancreatic malignanices in patients with intraductal papillary mucinous neoplasms of pancreas.

7. First degree family history of gastric cancer is significant risk factors for extrapancreatic malignanices in patients with intraductal papillary mucinous neoplasms of pancreas.

8. Further research are needed in order to fully enlighten the role of first degree family history of colorectal carcinoma as a risk factor for extrapancreatic malignanices in patients with intraductal papillary mucinous neoplasms of pancreas.

9. Intraductal papillary mucinous neoplasms of the pancreas are not associated with single nucleotide polymorphisms rs6983267, rs6993464, rs7014346, rs10505477 within human chromosomal region 8q24.

10. Further research are needed in order to examine if other single nucleotide polymorphisms within the human chromosomal region 8q24 are associated with intraductal papillary mucinous neoplasms of the pancreas.

11. Patients with intraductal papillary mucinous neoplasms of pancreas are not in an increased risk for development of adenomatous colorectal polyps.

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Biography

Nikola Panić was born in 1984 in Valjevo, Serbia where he finished elementary school and gymnasium. He was enrolled in School of Medicine, University of Belgrade in 2003 and finished medical studies in 2009 with average mark 9.42. He finished academic specialization in Digestive system in 2012 by presenting the thesis *"PCR in Helicobacter spp. diagnostic in extragastric malignancies of digestive system"*. He started doctoral studies in Epidemiology in 2011 in School of Medicine, University of Belgrade and conducted his PhD research at Catholic University, Rome, Italy as a participant in ERAWEB scholarship program supported by European Commission.

Since 2011 he works at the Digestive Endoscopy Unit at University Clinic "Dr Dragisa Misovic-Dedinje" in Belgrade, Serbia, currently as a resident in internal medicine. He has had professional training in gastroenterology and digestive endoscopy in renowned centers in Rome, Barcelona and Udine.

He received a National Scholar Award from United European Gastroenterology federation in 2011 and a Visiting Fellowship Grant in 2016 from the same organization. He is author and co-author of large number of scientific papers in fields of gastroenterology and epidemiology which were published and presented on scientific journals and conferences. He also authored two book chapters in field of endoscopic ultrasound.

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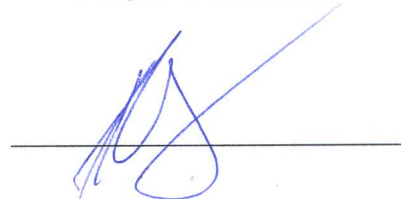
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