**Name of journal:** **World Journal of Gastroenterology**

**ESPS Manuscript NO: 12431**

**Columns: Review**

**Pancreatic cancer early detection: expanding higher-risk group with clinical and metabolomics parameters**

Urayama S. Pancreatic cancer early detection with new parameters

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**Author contributions:** Urayama S conceived and wrote the paper.

**Conflict-of-interest:** the author has no conflict of interest related to the manuscript.

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**Received:** July 8, 2014

**Peer-review started:** July 8, 2014

**First decision:** August 15, 2014

**Revised:** October 1, 2014

**Accepted:** January 8, 2015

**Article in press:**

**Published online:**

**Abstract**

Pancreatic ductal adenocarcinoma (PDAC) is the fourth and fifth leading cause of cancer death for each gender in developed countries. With lack of effective treatment and screening scheme available for the general population, the mortality rate is expected to increase over the next several decades in contrast to the other major malignancies such as lung, breast, prostate and colorectal cancers. Endoscopic ultrasound, with its highest level of detection capacity of smaller pancreatic lesions, is the commonly employed and preferred clinical imaging-based PDAC detection method. Various molecular biomarkers have been investigated for characterization of the disease, but none are shown to be useful or validated for clinical utilization for early detection. As seen from studies of a small subset of familial or genetically high-risk PDAC groups, the higher yield and utility of imaging-based screening methods are demonstrated for these groups. Multiple recent studies on the unique cancer metabolism including PDAC, demonstrate the potential for utility of the metabolites as the discriminant markers for this disease. In order to generate an early PDAC detection screening strategy available for a wider population, we propose to expand the population of higher risk PDAC group with combination clinical and metabolomics parameters.

**Key words:** Pancreatic cancer; Early detection; Endoscopic ultrasound; Metabolomics; Biomarkers

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**Core tip:** This is a summary of current pancreatic cancer cohort early detection studies and a potential approach being considered for future application. This is an area that requires heightened efforts as lack of effective treatment and screening scheme for wider population is leading this particular disease to be the second lethal cancer by 2030.

Urayama S. Pancreatic cancer early detection: expanding higher-risk group with clinical and metabolomics parameters. *World J Gastroenterol* 2015; In press

**Introduction**

Currently, pancreatic ductal adenocarcinoma (PDAC) is the fourth major cause of cancer mortality in the United States[1]. It is predicted that 46420 new cases and 39590 deaths would result from pancreatic cancer in the United States in 2014[2].Worldwide, there were 277668 new cases and 266029 deaths from this cancer in 2008[3].In comparison to other major malignancies such as breast, colon, lung and prostate cancers with their respective 89%, 64%, 16%, 99% 5-year survival rate, PDAC at 6% is conspicuously low[2]. For PDAC, the only curative option is surgical resection, which is applicable in only 10%–15% of patients due to the common discovery of late stage at diagnosis[4].In fact, PDAC is notorious for late stage discovery as evidenced by the low percentage of localized disease at diagnosis, compared to other malignancies: breast (61%), colon (40%), lung (16%), ovarian (19%), prostate (91%), and pancreatic cancer (7%)[5]. With the existing effective screening methods, the decreasing trends of cancer death rate are seen in major malignancies such as breast, prostate and colorectal cancer. In contrast, it is estimated that PDAC is expected to be surfacing as the second leading cause of cancer death by 2030[6].

With the distinct contribution of late-stage discovery and general lack of effective medical therapy, a critical approach in reversing the poor outcome of pancreatic cancer is to develop an early detection scheme for the tumor. In support of this, we see the trend that despite the poor prognosis of the disease, for those who have undergone curative resection with negative margins, the 5-year survival rate is 22% in contrast to 2% for the advanced-stage with distant metastasis[7,8].An earlier diagnosis with tumor less than 2 cm (T1) is associated with a better 5-year survival of 58% compared to 17% for stage IIB PDAC[9].Ariyama *et al*[10] reported complete survival of 79 patients with less than 1 cm tumors after surgical resection.Furthermore, as a recent report indicates, the estimated time from the transformation to pre-metastatic growths of pancreatic cancer is approximately 15 years[11]; there is a wide potential window of opportunity to apply developing technologies in early detection of this cancer.

In this article, we will review the recent studies on the PDAC early detection approaches and ongoing research endeavors in developing early detection schemes for this devastating disease, with specific attention to application of combined clinical and metabolomics parameters.

**PDAC Early Detection and Diagnosis – Imaging-based Tests**

Over the past few decades, endoscopic ultrasound (EUS) has proven itself to be a superior imaging study for detection of a small or early-stage pancreatic neoplasm as compared to other modalities such as transabdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography scans and angiography[12-14].Yasuda *et al*[15] and Rosch *et al*[16] had initially demonstrated the superiority of EUS in detection of small pancreatic lesions.More recently, DeWitt *et al*[17] had verified the superiority of EUS as compared to multi-detector CT scan.In another study, Khashab *et al*[18] demonstrated that the sensitivity of EUS in detecting a pancreatic mass was significantly greater than that of CT images, and particularly for pancreatic neuroendocrine tumors, which commonly consist of smaller lesions.In addition, EUS detected CT-negative tumors in more than 90% of the cases. As an additional diagnostic modality, EUS-guided fine needle aspiration (FNA) provides success rates of 90%–95%, with an overall sensitivity and specificity of 85%-90% and 98%-99%, respectively[19-22].Thus, the utility and the advantage of EUS enable visualization and targeting of small pancreatic masses. Lesions of 5 mm or less could be visualized and sampled, which might not have been accessible or identifiable by other imaging modalities[23].

**Diagnostic Molecular Markers and Pancreatic Cancer**

In order to enhance the diagnostic accuracy of PDAC, molecular markers on EUS-FNA samples have been evaluated in recent years. Utilities of DNA mutations and loss of heterozygosity are being reported as potential surrogate markers of the cancer[24,25].In a recent study, Takahashi *et al*[26] assessed *k-ras* point mutations in PDAC and chronic focal pancreatitis samples obtained by EUS-FNA[27,28].The study revealed the presence of point mutations of *k-ras* in 74% of patients with PDAC compared to no mutations in chronic focal pancreatitis. In another study, Tada *et al*[29] reported a high *k-ras* gene mutation rate in 20 of 26 cases of EUS-FNA specimens (77%) and in 12 of 19 cases of pancreatic juice (63%) in PDAC.However, the presence of *k-ras* mutations in a benign condition such as chronic pancreatitis and premalignant lesions such as intraductal papillary mucinous neoplasm (IPMN) in addition to lack of such mutations in 20% of PDAC limit the usage of this test solely as a diagnostic or a detection tool. Other studies analyzing p53 by immunohistochemistry[30],telomerase activity with a ribonucleoprotein enzyme[31],and a broad panel of microsatellite allele loss markers demonstrated similar results[32].In the presence of inconclusive EUS-FNA cytology, molecular markers could potentially complement EUS-FNA cytology results to help establish the diagnosis of malignancy.

**Select Population-based Research for Early Detection Scheme Development**

***PDAC screening in high-risk individuals***

Currently, a general population-screening program for PDAC is not cost-effective because of low relative disease incidence and non-availability of simple, cheap, highly accurate non-invasive tests. The main goal of the screening is to identify clinically significant precursor or early stage PDAC. However, since overwhelming majority of premalignant and small PDAC lesions is asymptomatic, we do not have a definite surrogate marker to identify a subset population for screening. Consequently, as one of the approaches in investigating the risks, research has focused on identification of a subset of individuals with a higher-risk for PDAC development in order to elucidate the genetic predilection. Up to 10% of PDAC patients have a familial/genetic basis and they have increased risk of developing both pancreatic and extra-pancreatic malignancies[33-37].Classic categorization of high-risk patients are based on the highly associated genetic risks defined as those who have significant family history of the cancer or have an inherited PDAC syndrome with a known genetic abnormality (Table 1).

**Familial pancreatic cancer:** Familial pancreatic cancer (FPC) cohort (cancer in two or more first-degree relatives (FDRs) or in three or more affected family members - including one first-degree relative) is considered a high-risk and a candidate for screening program[47,54,55].Currently, the genetic foundation for FPC is not fully understood. Various investigations have demonstrated the presence of a germline mutation in the *BRCA2* gene[47,48,49], association of BRCA1[46,56], *paladin* gene mutation[57] as well as other genes such as apolipoprotein A4, CEA, keratin 19, stratifin, trefoil factor, and S100A6[58,59] in FPC, and more recently identification of PALB2[60],as a pancreatic cancer susceptibility gene. These facts suggest that multiple and heterogeneous factors are likely at play for the genesis of PDAC in this subset.

Analysis of the PDAC kindred data from Johns Hopkins’ National Familial Pancreas Tumor Registry (NFPTR) has shown that the risk of PDAC in individuals with two afflicted FDRs is 6.4% and the lifetime risk is 8%-12%; for persons with three afflicted FDRs, the relative and lifetime risks for PDAC and increase to 32% and 16%-32%, respectively[36].Brune *et al*[61] in their recent article reported a higher risk of PDAC among FPC kindred with a younger onset (age less than 50).Rulyak *et al*[62] in another study found smoking as a significant risk factor in FPC cohort, especially among males and those under age 50. This factor increases the cancer risk by 2.0-3.7 times and lowers the onset age by 10 years.A risk assessment software tool, PancPRO, has been generated and is available for calculating the risk for individuals with familial pancreatic cancer (<http://www4.utsouthwestern.edu/breasthealth/cagene/default.asp>)[35].

**Screening modalities and the current screening programs:** Many of the screening programs have used additional investigational biomarker to complement imaging tests to identify the early lesions. A commonly used marker, CA19-9, is neither sensitive nor specific independently for early PDAC or precursor detection. Kim *et al*[63] in their study found only 0.9% positive-predictive value using the standard cut-off value (37 U/mL).Other biomarkers investigated recently include MIC-1, CEACAM-1, SPan1, DUPAN, Alpha4GNT, and PAM4, but none is validated for routine clinical use[64].In another approach, elevated fasting-glucose level has been demonstrated to be associated with for sporadic PDAC[65] and is currently utilized by an European registry in high-risk people with mutational analysis of pancreatic juice along with *p16* promoter methylation status.

Several international screening programs exist for PDAC in high-risk individuals. “Cancer of the Pancreas Screening Study” (CAPS study), led by John Hopkins University, is the largest screening program that involves 24 American Centers of Excellence. To date, three studies, CAPS 1, CAPS 2 and CAPS 3, have been completed. (Table 2)

In the CAPS 1, thirty-eight patients including 31 from a kindred with > 3 affected with PDAC, 6 with 2 affected relatives, and 1 patient with Peutz-Jeghers syndrome (PJS) were studied. Screening protocol with EUS revealed six pancreatic masses: 1 invasive PDAC, 1 benign IPMN, 2 serous cystadenomas, and 2 non-neoplastic lesions. The yield of screening was 5.3% in this study[66].In the CAPS 2, seventy-eight high-risk patients were studied[67].In 8 patients, the screening found pancreatic neoplasia, confirmed by surgery or FNA: 6 benign IPMNs, 1 IPMN that progressed to invasive PDAC, and another had high-grade pancreatic intraepithelial neoplasia (PanIN-3). The CAPS 3 was a multicenter prospective cohort study involving annual EUS, MRI screening with assays of DNA and protein markers in serum and pancreatic juice. Over 200 patients were enrolled over a three-year period. The study results on the detection modality comparison demonstrated that the EUS has the highest rate of detection of early neoplastic changes in up to 42.6% of the asymptomatic high-risk group[68].

In another study from the University of Washington, high-risk familial cohorts were screened with EUS, beginning 10 years prior to the earliest index PDAC case. If EUS was normal, they underwent a repeat EUS in 2-3 years. With abnormal EUS findings, they were referred for ERCP and if abnormalities were noted, patients were offered surgical intervention[69].Among 75 screened subjects, 15 had gone to surgery for abnormal EUS and ERCP findings. All surgical cases revealed premalignant lesions: PanIN-3 in 10 and PanIN-2 in five cases[70].The study gave a yield of 13% (10 out of 75) for detecting PanIN-3 lesions. A single patient developed unresectable PDAC during the surveillance.

In a German PDAC screening program (FaPaCa), 76 patients were followed using annual EUS, MRCP, and laboratory assays (*CDKN2a* and *BRCA2* genetic analysis, CA19-9 and CEA). Any appreciable lesion was evaluated with EUS. With an abnormal finding, the patient underwent surgical exploration and if malignancy was detected, total pancreatectomy was performed. In 10 cases, lesions were seen on EUS as compared to only seven detected by MR scan. Out of the seven MRCP-positive cases, six underwent resections and the histology showed one PanIN-3, one PanIN-2, one PanIN-1, and three with other benign lesions. This resulted in a diagnostic yield of 1.3% for PanIN-3 detection[71].Another study from the Netherlands in 44 high-risk subjects demonstrated a 7% detection rate for asymptomatic PDAC and a 16% for premalignant lesions[72].

The International CAPS Consortium have recently met and reported a suggested guideline for current PDAC screening based on the risk[73].A consensus (≥ 75% agreement by the participants) was reached that the following groups should be offered screening (only to individuals who are surgical candidate): (1) FDRs of the cancer patients from a familial pancreatic cancer cohort with at least two affected FDRs; (2) patients with Peutz-Jeghers syndrome; and (3) *p16*, *BRCA2* and hereditary non-polyposis colorectal cancer mutation carriers with at least single affected FDR. The initial screening should include EUS and/or MRI. However, consensus was not reached on the beginning and the end age of screening/surveillance and the interval of the examination. Their conclusions also included requirement for further studies, and the clinical management should occur at high-volume centers with multidisciplinary teams.

**Future of Pancreatic Cancer Screening**

Current screening programs have demonstrated that the EUS evaluation can detect premalignant lesions and early cancers in certain small subset of high-risk groups. However, as the overwhelming majority of PDAC cases involve patients who develop the disease sporadically without a recognized genetic abnormality, the application of this modality for PDAC detection screening is very limited for the general adult population.

***Select population based approach***

**identification of a higher-PDAC-risk group:** As the prevalence of PDAC in the general United States population over the age 55 is approximately 68 per 100000, a candidate discriminant test with a specificity of 98% and a sensitivity of 100% would generate 1999 false-positive test results and 68 true-positives[74]. Thus, relying on a single determinant for distinguishing the PDAC early-stage cases from the general population would necessitate a highly accurate test with a specificity of greater than 99%. More practical approach, then, would be to begin with a subset of population with a higher prevalence, and in conjunction with novel surrogate markers to curtail the at-risk subset, we could begin to identify the group with significantly increased PDAC risk for whom the endoscopic/imaging-based screening strategy could be applied.

An initial approach in selection of the screening population is to utilize selective clinical parameters that could be used to curtail the subset of the general population at increased PDAC risk. For instance, based on the epidemiological evidence, such clinical parameters include hyperglycemia or diabetes, which are noted in 50%-80% of pancreatic cancer patients[75-79]. Though not encompassing all PDAC patients, this subset includes a much larger proportion of PDAC patients for whom we may select further for screening. Similarly, patients with a history of chronic pancreatitis or obesity are reported to have increased PDAC risk during their lifetime[80-85]. Recent findings from molecular biology and animal studies investigating effects of diet-induced obesity in a PDAC mouse model demonstrated increased occurrence of pancreatic inflammation and accelerated pancreatic neoplastic changes, supporting the association of obesity and pancreatic inflammation and PDAC risks[86,87]. Considering that millions are being diagnosed with diabetes or glucose intolerance, chronic pancreatitis, or obesity annually in comparison to PDAC, however, further refinement of the screening patient group is critically needed to justify for developing a larger scale screening protocol.

***Translational research - application of metabolomics approach***

Initially established as a key methodology in the field of inborn metabolic errors and toxicology, metabolomics have developed over the years to examine a much wider array of low-molecular-weight products or intermediates within the biological state of a cell, tissue, organ, or organism. A metabolome represents a physiological readout of the biochemical state in an individual’s body compartment, and provides the functional terminal signals of the genome and proteome, reflecting more closely the current phenotypic state of an individual in response to the environmental stimuli[88]. Thus, metabolomics data has considerable potential in elucidating cancer-development risks, with its additional capacity for providing temporal molecular information to the ongoing changes originating from genetic PDAC risk alone.

With the recent advancement in the technology and resumed interest in the cancer-associated metabolic abnormality[89,90],application of metabolomics in the cancer field has attracted more attention[91]. Cancer-related metabolic reprogramming, Warburg effect, has been known since nearly a century ago in association with various solid tumors including PDAC[92], as cancer cells undergo energetically inefficient glycolysis even in the presence of oxygen in the environment (aerobic glycolysis)[93].A number of common cancer mutations including Akt1, HIF (hypoxia-inducible factor), and p53 have been shown to support the Warburg effect through glycolysis and down-regulation of metabolite flux through the Krebs cycle[94-101]. In PDAC, increased phosphorylation or activation of Akt1 has also been reported (illuminating on the importance of enzyme functionality)[102] as well as involvement of HIF1 in the tumor growth via effects on glycolytic process[103,104] and membrane-bound glycoprotein (MUC17) regulation[105] – reflective of activation of metabolic pathways. Further evidences of loss-of-function genetic mutations in key mitochondrial metabolic enzymes such as succinate dehydrogenase and fumarate hydratase, isocitrate dehydrogenase, phosphoglycerate dehydrogenase support carcinogenesis and the Warburg effect[106-110]. Other important alternative pathways in cancer metabolism such as glutaminolysis and pyruvate kinase isoform suppression have been shown to accumulate respective upstream intermediates and reduction of associated end products such as NADPH, ribose-5-phosphate and nucleic acids[111-116].As such, various groups have reported metabolomics biomarker applications for different cancers[117,118].

As a major organ involved in metabolic regulation in a healthy individual, pancreatic disorder such as malignancy is anticipated to influence the normal metabolism, presenting further rationale and interest in elucidating the implication of malignant transformation and PDAC development. Proteomic analysis of the pancreatic cancer cells demonstrated alteration in proteins involved in metabolic pathways including increased expression of glycolytic and reduced Krebs cycle enzymes, and accumulation of key proteins involved in glutamine metabolism, in support of Warburg effect. These in turn play significant role in nucleotide and amino acid biosynthesis required for sustaining the proliferating cancer cells[119].Applications of sensitive mass spectrometric techniques in metabolomics study of PDAC detection biomarkers have led to identification of a set of small molecules or metabolites (or biochemical intermediates) that are potent discriminants of developing PDAC and the controls (See Figure 1 as an example of metabolomics based analysis, allowing segregation of PDAC from benign cases). Recent reports from our group as well as others have demonstrated that specific candidate metabolites consisting of amino acids, bile acids, and a number of lipids and fatty acids – suspected to be reflective of tumor proliferation as well as many systemic response yet to be determined - were identified as potential discriminant for blood-based PDAC biomarkers[120-123].As a further supporting data, elucidation of lipids and fatty acids as discriminant factors from PDAC and benign lesions from the cancer tissue and adjacent normal tissue has been reported recently[124].

By virtue of simultaneously depicting the multiple metabolite levels, metabolomics approach reveals various biochemical pathways that are uniquely involved in malignant conditions and has led to findings such as abnormalities of glycine and its mitochondrial biosynthetic pathway, as a potential therapeutic target in certain cancers[125].Moreover, in combination with other systems biology approaches such as transcriptomics and proteomics, further refinement in characterization of cancer development and therapeutic targets as well as identification of potential biomarkers could be realized for PDAC. Since many enzymes in a metabolic network determine metabolites’ level and nonlinear quantitative relationship from the genes to the proteome and metabolome levels exist, a metabolome cannot be easily decomposed to a specific single marker, which will designate the cancer state[126].Thus, in order to delineate a pathological state such as PDAC, multiple metabolomic features might be required for accurate depiction of a developing cancer. Future studies are anticipated to incorporate cancer systems’ biological knowledge, including metabolomics, for optimal designation of PDAC biomarkers, which would be utilized in conjunction with a clinical-parameter-derived population subset for establishing the PDAC screening population. Subsequently, further validation studies for the PDAC biomarkers need to be performed.

**Conclusion**

Current imaging-based detection and diagnostic methods for PDAC is effectively providing answers to clinical questions raised for patients with signs or symptoms of suspected pancreatic lesions. However, the endoscopic/imaging-based screening schemes are currently limited in applications to early PDAC detection in asymptomatic patients, aside from a small group of known genetically high-risk groups. There is a high demand for developing a method of selecting distinct subsets among the general population for implementing the endoscopic/imaging screening test effectively. Application of combinations of clinical risk parameters/factors with the developing molecular biomarkers from translational science such as metabolomics analysis brings hopes of providing us with early PDAC detection markers, and developing effective early detection screening scheme for the patients in the near future.

**REFERENCES**

1 **National Cancer Institute**. Cancer topics: Pancreatic cancer. Available from: URL: http://www.cancer.gov/cancertopics/types/pancreatic

2 **American Cancer Society.** Cancer Facts & Figures, 2014. Available from URL: http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/index

3 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

4 **Beger HG**, Rau B, Gansauge F, Poch B, Link KH. Treatment of pancreatic cancer: challenge of the facts. *World J Surg* 2003; **27**: 1075-1084 [PMID: 12925907 DOI: 10.1007/s00268-003-7165-7]

5 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

6 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472]

7 **Ahmad NA**, Lewis JD, Ginsberg GG, Haller DG, Morris JB, Williams NN, Rosato EF, Kochman ML. Long term survival after pancreatic resection for pancreatic adenocarcinoma. *Am J Gastroenterol* 2001; **96**: 2609-2615 [PMID: 11569683 DOI: 10.1111/j.1572-0241.2001.04123.x]

8 **Howard TJ**, Krug JE, Yu J, Zyromski NJ, Schmidt CM, Jacobson LE, Madura JA, Wiebke EA, Lillemoe KD. A margin-negative R0 resection accomplished with minimal postoperative complications is the surgeon's contribution to long-term survival in pancreatic cancer. *J Gastrointest Surg* 2006; **10**: 1338-145; discussion 1338-145; [PMID: 17175452 DOI: 10.1016/j.gassur.2006.09.008]

9 **Egawa S**, Takeda K, Fukuyama S, Motoi F, Sunamura M, Matsuno S. Clinicopathological aspects of small pancreatic cancer. *Pancreas* 2004; **28**: 235-240 [PMID: 15084963 DOI: 10.1097/00006676-200404000-00004]

10 **Ariyama J**, Suyama M, Ogawa K, Ikari T. [Screening of pancreatic neoplasms and the diagnostic rate of small pancreatic neoplasms]. *Nihon Rinsho* 1986; **44**: 1729-1734 [PMID: 3537369]

11 **Yachida S**, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010; **467**: 1114-1117 [PMID: 20981102 DOI: 10.1038/nature09515]

12 **Rösch T**, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992; **102**: 188-199 [PMID: 1727753]

13 **Müller MF**, Meyenberger C, Bertschinger P, Schaer R, Marincek B. Pancreatic tumors: evaluation with endoscopic US, CT, and MR imaging. *Radiology* 1994; **190**: 745-751 [PMID: 8115622 DOI: 10.1148/radiology.190.3.8115622]

14 **Agarwal B**, Abu-Hamda E, Molke KL, Correa AM, Ho L. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. *Am J Gastroenterol* 2004; **99**: 844-850 [PMID: 15128348 DOI: 10.1111/j.1572-0241.2004.04177.x]

15 **Yasuda K**, Mukai H, Fujimoto S, Nakajima M, Kawai K. The diagnosis of pancreatic cancer by endoscopic ultrasonography. *Gastrointest Endosc* 1988; **34**: 1-8 [PMID: 3280392 DOI: 10.1016/S0016-5107(88)71220-1]

16 **Rösch T**, Lorenz R, Braig C, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Endoscopic ultrasound in pancreatic tumor diagnosis. *Gastrointest Endosc* 1991; **37**: 347-352 [PMID: 2070987 DOI: 10.1016/S0016-5107(91)70729-3]

17 **DeWitt J**, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004; **141**: 753-763 [PMID: 15545675 DOI: 10.7326/0003-4819-141-10-200411160-00006]

18 **Khashab MA**, Yong E, Lennon AM, Shin EJ, Amateau S, Hruban RH, Olino K, Giday S, Fishman EK, Wolfgang CL, Edil BH, Makary M, Canto MI. EUS is still superior to multidetector computerized tomography for detection of pancreatic neuroendocrine tumors. *Gastrointest Endosc* 2011; **73**: 691-696 [PMID: 21067742 DOI: 10.1016/j.gie.2010.08.030]

19 **Vilmann P**, Jacobsen GK, Henriksen FW, Hancke S. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. *Gastrointest Endosc* 1992; **38**: 172-173 [PMID: 1568614 DOI: 10.1016/S0016-5107(92)70385-X]

20 **Hewitt MJ**, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. *Gastrointest Endosc* 2012; **75**: 319-331 [PMID: 22248600 DOI: 10.1016/j.gie.2011.08.049]

21 **Hébert-Magee S**, Bae S, Varadarajulu S, Ramesh J, Frost AR, Eloubeidi MA, Eltoum IA. The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis. *Cytopathology* 2013; **24**: 159-171 [PMID: 23711182 DOI: 10.1111/cyt.12071]

22 **Luz LP**, Al-Haddad MA, Sey MS, DeWitt JM. Applications of endoscopic ultrasound in pancreatic cancer. *World J Gastroenterol* 2014; **20**: 7808-7818 [PMID: 24976719 DOI: 10.3748/wjg.v20.i24.7808]

23 **Fritscher-Ravens A**, Izbicki JR, Sriram PV, Krause C, Knoefel WT, Topalidis T, Jaeckle S, Thonke F, Soehendra N. Endosonography-guided, fine-needle aspiration cytology extending the indication for organ-preserving pancreatic surgery. *Am J Gastroenterol* 2000; **95**: 2255-2260 [PMID: 11007226 DOI: 10.1111/j.1572-0241.2000.02311.x]

24 **Pellisé M**, Castells A, Ginès A, Solé M, Mora J, Castellví-Bel S, Rodríguez-Moranta F, Fernàndez-Esparrach G, Llach J, Bordas JM, Navarro S, Piqué JM. Clinical usefulness of KRAS mutational analysis in the diagnosis of pancreatic adenocarcinoma by means of endosonography-guided fine-needle aspiration biopsy. *Aliment Pharmacol Ther* 2003; **17**: 1299-1307 [PMID: 12755843 DOI: 10.1046/j.1365-2036.2003.01579.x]

25 **Goggins M**. Molecular markers of early pancreatic cancer. *J Clin Oncol* 2005; **23**: 4524-4531 [PMID: 16002843 DOI: 10.1200/JCO.2005.19.711]

26 **Takahashi K**, Yamao K, Okubo K, Sawaki A, Mizuno N, Ashida R, Koshikawa T, Ueyama Y, Kasugai K, Hase S, Kakumu S. Differential diagnosis of pancreatic cancer and focal pancreatitis by using EUS-guided FNA. *Gastrointest Endosc* 2005; **61**: 76-79 [PMID: 15672060 DOI: 10.1016/S0016-5107(04)02224-2]

27 **Maluf-Filho F**, Kumar A, Gerhardt R, Kubrusly M, Sakai P, Hondo F, Matuguma SE, Artifon E, Monteiro da Cunha JE, César Machado MC, Ishioka S, Forero E. Kras mutation analysis of fine needle aspirate under EUS guidance facilitates risk stratification of patients with pancreatic mass. *J Clin Gastroenterol* 2007; **41**: 906-910 [PMID: 18090159 DOI: 10.1097/mcg.0b013e31805905e9]

28 **Bournet B**, Souque A, Senesse P, Assenat E, Barthet M, Lesavre N, Aubert A, O'Toole D, Hammel P, Levy P, Ruszniewski P, Bouisson M, Escourrou J, Cordelier P, Buscail L. Endoscopic ultrasound-guided fine-needle aspiration biopsy coupled with KRAS mutation assay to distinguish pancreatic cancer from pseudotumoral chronic pancreatitis. *Endoscopy* 2009; **41**: 552-557 [PMID: 19533561 DOI: 10.1055/s-0029-1214717]

29 **Tada M**, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N, Shiratori Y, Omata M. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. *Am J Gastroenterol* 2002; **97**: 2263-2270 [PMID: 12358243 DOI: 10.1111/j.1572-0241.2002.05980.x]

30 **Itoi T**, Takei K, Sofuni A, Itokawa F, Tsuchiya T, Kurihara T, Nakamura K, Moriyasu F, Tsuchida A, Kasuya K. Immunohistochemical analysis of p53 and MIB-1 in tissue specimens obtained from endoscopic ultrasonography-guided fine needle aspiration biopsy for the diagnosis of solid pancreatic masses. *Oncol Rep* 2005; **13**: 229-234 [PMID: 15643503 DOI: 10.3892/or.13.2.229]

31 **Mishra G**, Zhao Y, Sweeney J, Pineau BC, Case D, Ho C, Blackstock AW, Geisinger K, Howerton R, Levine E, Shen P, Ibdah J. Determination of qualitative telomerase activity as an adjunct to the diagnosis of pancreatic adenocarcinoma by EUS-guided fine-needle aspiration. *Gastrointest Endosc* 2006; **63**: 648-654 [PMID: 16564867 DOI: 10.1016/j.gie.2005.11.056]

32 **Salek C**, Benesova L, Zavoral M, Nosek V, Kasperova L, Ryska M, Strnad R, Traboulsi E, Minarik M. Evaluation of clinical relevance of examining K-ras, p16 and p53 mutations along with allelic losses at 9p and 18q in EUS-guided fine needle aspiration samples of patients with chronic pancreatitis and pancreatic cancer. *World J Gastroenterol* 2007; **13**: 3714-3720 [PMID: 17659731 DOI: 10.3748/wjg.v13.i27.3714]

33 **Shi C**, Hruban RH, Klein AP. Familial pancreatic cancer. *Arch Pathol Lab Med* 2009; **133**: 365-374 [PMID: 19260742 DOI: 10.1043/1543-2165-133.3.365]

34 **Permuth-Wey J**, Egan KM. Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. *Fam Cancer* 2009; **8**: 109-117 [PMID: 18763055 DOI: 10.1007/s10689-008-9214-8]

35 **Wang W**, Chen S, Brune KA, Hruban RH, Parmigiani G, Klein AP. PancPRO: risk assessment for individuals with a family history of pancreatic cancer. *J Clin Oncol* 2007; **25**: 1417-1422 [PMID: 17416862 DOI: 10.1200/JCO.2006.09.2452]

36 **Klein AP**, Brune KA, Petersen GM, Goggins M, Tersmette AC, Offerhaus GJ, Griffin C, Cameron JL, Yeo CJ, Kern S, Hruban RH. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res* 2004; **64**: 2634-2638 [PMID: 15059921 DOI: 10.1158/0008-5472.CAN-03-3823]

37 **Wang L**, Brune KA, Visvanathan K, Laheru D, Herman J, Wolfgang C, Schulick R, Cameron JL, Goggins M, Hruban RH, Klein AP. Elevated cancer mortality in the relatives of patients with pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2829-2834 [PMID: 19843679 DOI: 10.1158/1055-9965.EPI-09-0557]

38 **Giardiello FM**, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, Cruz-Correa M, Offerhaus JA. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 2000; **119**: 1447-1453 [PMID: 11113065 DOI: 10.1053/gast.2000.20228]

39 **Rebours V**, Boutron-Ruault MC, Schnee M, Férec C, Maire F, Hammel P, Ruszniewski P, Lévy P. Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series. *Am J Gastroenterol* 2008; **103**: 111-119 [PMID: 18184119 DOI: 10.1111/j.1572-0241.2007.01597.x]

40 **Lowenfels AB**, Maisonneuve P, DiMagno EP, Elitsur Y, Gates LK, Perrault J, Whitcomb DC. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst* 1997; **89**: 442-446 [PMID: 9091646 DOI: 10.1093/jnci/89.6.442]

41 **Raimondi S**, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract Res Clin Gastroenterol* 2010; **24**: 349-358 [PMID: 20510834 DOI: 10.1016/j.bpg.2010.02.007]

42 **Goldstein AM**, Fraser MC, Struewing JP, Hussussian CJ, Ranade K, Zametkin DP, Fontaine LS, Organic SM, Dracopoli NC, Clark WH. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med* 1995; **333**: 970-974 [PMID: 7666916 DOI: 10.1056/NEJM199510123331504]

43 **Whelan AJ**, Bartsch D, Goodfellow PJ. Brief report: a familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor gene. *N Engl J Med* 1995; **333**: 975-977 [PMID: 7666917 DOI: 10.1056/NEJM199510123331505]

44 **Klein AP**. Genetic susceptibility to pancreatic cancer. *Mol Carcinog* 2012; **51**: 14-24 [PMID: 22162228 DOI: 10.1002/mc.20855]

45 **van Asperen CJ**, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, Ausems MG, Menko FH, Gomez Garcia EB, Klijn JG, Hogervorst FB, van Houwelingen JC, van't Veer LJ, Rookus MA, van Leeuwen FE. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet* 2005; **42**: 711-719 [PMID: 16141007 DOI: 10.1136/jmg.2004.028829]

46 **Brose MS**, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL, Weber BL. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *J Natl Cancer Inst* 2002; **94**: 1365-1372 [PMID: 12237282 DOI: 10.1093/jnci/94.18.1365]

47 **Couch FJ**, Johnson MR, Rabe KG, Brune K, de Andrade M, Goggins M, Rothenmund H, Gallinger S, Klein A, Petersen GM, Hruban RH. The prevalence of BRCA2 mutations in familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 342-346 [PMID: 17301269 DOI: 10.1158/1055-9965.EPI-06-0783]

48 **Goggins M**, Schutte M, Lu J, Moskaluk CA, Weinstein CL, Petersen GM, Yeo CJ, Jackson CE, Lynch HT, Hruban RH, Kern SE. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res* 1996; **56**: 5360-5364 [PMID: 8968085]

49 **Hahn SA**, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, Gerdes B, Kress R, Ziegler A, Raeburn JA, Campra D, Grützmann R, Rehder H, Rothmund M, Schmiegel W, Neoptolemos JP, Bartsch DK. BRCA2 germline mutations in familial pancreatic carcinoma. *J Natl Cancer Inst* 2003; **95**: 214-221 [PMID: 12569143 DOI: 10.1093/jnci/95.3.214]

50 **Lal G**, Liu G, Schmocker B, Kaurah P, Ozcelik H, Narod SA, Redston M, Gallinger S. Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. *Cancer Res* 2000; **60**: 409-416 [PMID: 10667595]

51 **Murphy KM**, Brune KA, Griffin C, Sollenberger JE, Petersen GM, Bansal R, Hruban RH, Kern SE. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. *Cancer Res* 2002; **62**: 3789-3793 [PMID: 12097290]

52 **Kastrinos F**, Mukherjee B, Tayob N, Wang F, Sparr J, Raymond VM, Bandipalliam P, Stoffel EM, Gruber SB, Syngal S. Risk of pancreatic cancer in families with Lynch syndrome. *JAMA* 2009; **302**: 1790-1795 [PMID: 19861671 DOI: 10.1001/jama.2009.1529]

53 **McWilliams R**, Highsmith WE, Rabe KG, de Andrade M, Tordsen LA, Holtegaard LM, Petersen GM. Cystic fibrosis transmembrane regulator gene carrier status is a risk factor for young onset pancreatic adenocarcinoma. *Gut* 2005; **54**: 1661-1662 [PMID: 16227367 DOI: 10.1136/gut.2005.074534]

54 **Greenhalf W**, Grocock C, Harcus M, Neoptolemos J. Screening of high-risk families for pancreatic cancer. *Pancreatology* 2009; **9**: 215-222 [PMID: 19349734 DOI: 10.1159/000210262]

55 **Brand RE**, Lerch MM, Rubinstein WS, Neoptolemos JP, Whitcomb DC, Hruban RH, Brentnall TA, Lynch HT, Canto MI. Advances in counselling and surveillance of patients at risk for pancreatic cancer. *Gut* 2007; **56**: 1460-1469 [PMID: 17872573 DOI: 10.1136/gut.2006.108456]

56 **Lynch HT**, Deters CA, Snyder CL, Lynch JF, Villeneuve P, Silberstein J, Martin H, Narod SA, Brand RE. BRCA1 and pancreatic cancer: pedigree findings and their causal relationships. *Cancer Genet Cytogenet* 2005; **158**: 119-125 [PMID: 15796958 DOI: 10.1016/j.cancergencyto.2004.01.032]

57 **Pogue-Geile KL**, Chen R, Bronner MP, Crnogorac-Jurcevic T, Moyes KW, Dowen S, Otey CA, Crispin DA, George RD, Whitcomb DC, Brentnall TA. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. *PLoS Med* 2006; **3**: e516 [PMID: 17194196 DOI: 10.1371/journal.pmed.0030516]

58 **Earl J**, Yan L, Vitone LJ, Risk J, Kemp SJ, McFaul C, Neoptolemos JP, Greenhalf W, Kress R, Sina-Frey M, Hahn SA, Rieder H, Bartsch DK. Evaluation of the 4q32-34 locus in European familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1948-1955 [PMID: 17035404 DOI: 10.1158/1055-9965.EPI-06-0376]

59 **Zervos EE**, Tanner SM, Osborne DA, Bloomston M, Rosemurgy AS, Ellison EC, Melvin WS, de la Chapelle A. Differential gene expression in patients genetically predisposed to pancreatic cancer. *J Surg Res* 2006; **135**: 317-322 [PMID: 16815451 DOI: 10.1016/j.jss.2006.03.022]

60 **Jones S**, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, Lin JC, Palmisano E, Brune K, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Parmigiani G, Kern SE, Velculescu VE, Kinzler KW, Vogelstein B, Eshleman JR, Goggins M, Klein AP. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009; **324**: 217 [PMID: 19264984 DOI: 10.1126/science.1171202]

61 **Brune KA**, Lau B, Palmisano E, Canto M, Goggins MG, Hruban RH, Klein AP. Importance of age of onset in pancreatic cancer kindreds. *J Natl Cancer Inst* 2010; **102**: 119-126 [PMID: 20068195 DOI: 10.1093/jnci/djp466]

62 **Rulyak SJ**, Lowenfels AB, Maisonneuve P, Brentnall TA. Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. *Gastroenterology* 2003; **124**: 1292-1299 [PMID: 12730869 DOI: 10.1016/S0016-5085(03)00272-5]

63 **Kim JE**, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol* 2004; **19**: 182-186 [PMID: 14731128 DOI: 10.1111/j.1440-1746.2004.03219.x]

64 **Bussom S**, Saif MW. Methods and rationale for the early detection of pancreatic cancer. Highlights from the "2010 ASCO Gastrointestinal Cancers Symposium". Orlando, FL, USA. January 22-24, 2010. *JOP* 2010; **11**: 128-130 [PMID: 20208319]

65 **Stolzenberg-Solomon RZ**, Graubard BI, Chari S, Limburg P, Taylor PR, Virtamo J, Albanes D. Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. *JAMA* 2005; **294**: 2872-2878 [PMID: 16352795 DOI: 10.1001/jama.294.22.2872]

66 **Canto MI**, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM, Hruban RH. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. *Clin Gastroenterol Hepatol* 2004; **2**: 606-621 [PMID: 15224285 DOI: 10.1016/S1542-3565(04)00244-7]

67 **Canto MI**, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, Fishman EK, Brune K, Axilbund J, Griffin C, Ali S, Richman J, Jagannath S, Kantsevoy SV, Kalloo AN. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clin Gastroenterol Hepatol* 2006; **4**: 766-81; quiz 665 [PMID: 16682259 DOI: 10.1016/j.cgh.2006.02.005]

68 **Canto MI**, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, Syngal S, Saltzman JR, Mortele KJ, Lee J, Tamm E, Vikram R, Bhosale P, Margolis D, Farrell J, Goggins M. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. *Gastroenterology* 2012; **142**: 796-804; quiz e14-5 [PMID: 22245846 DOI: 10.1053/j.gastro.2012.01.005]

69 **Kimmey MB**, Bronner MP, Byrd DR, Brentnall TA. Screening and surveillance for hereditary pancreatic cancer. *Gastrointest Endosc* 2002; **56**: S82-S86 [PMID: 12297755 DOI: 10.1016/S0016-5107(02)70092-8]

70 **Carlson C**, Greenhalf W, Brentnall TA. Screening of hereditary pancreatic cancer families, in The Pancreas: An Integrated Textbook of Basic Science, Medicine and Surgery. Second Edition. In: Berger HG ,Bucher M, Kozarek R, Lerch M, Neoptolemos J, Warshaw A, Whitcomb D, Shiratori K, editors. Oxford UK: Wiley-Blackwell Publishing, 2008: 636-642 [DOI: 10.1002/9781444300123.ch64]

71 **Langer P**, Kann PH, Fendrich V, Habbe N, Schneider M, Sina M, Slater EP, Heverhagen JT, Gress TM, Rothmund M, Bartsch DK. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. *Gut* 2009; **58**: 1410-1418 [PMID: 19470496 DOI: 10.1136/gut.2008.171611]

72 **Poley JW**, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, van Eijck CH, Cats A, Kuipers EJ, Nio Y, Fockens P, Bruno MJ. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. *Am J Gastroenterol* 2009; **104**: 2175-2181 [PMID: 19491823 DOI: 10.1038/ajg.2009.276]

73 **Canto MI**, Harinck F, Hruban RH, Offerhaus GJ, Poley JW, Kamel I, Nio Y, Schulick RS, Bassi C, Kluijt I, Levy MJ, Chak A, Fockens P, Goggins M, Bruno M. International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut* 2013; **62**: 339-347 [PMID: 23135763 DOI: 10.1136/gutjnl-2012-303108]

74 **Lennon AM**, Wolfgang CL, Canto MI, Klein AP, Herman JM, Goggins M, Fishman EK, Kamel I, Weiss MJ, Diaz LA, Papadopoulos N, Kinzler KW, Vogelstein B, Hruban RH. The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? *Cancer Res* 2014; **74**: 3381-3389 [PMID: 24924775 DOI: 10.1158/0008-5472.CAN-14-0734]

75 **Chari ST**, Leibson CL, Rabe KG, Timmons LJ, Ransom J, de Andrade M, Petersen GM. Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. *Gastroenterology* 2008; **134**: 95-101 [PMID: 18061176 DOI: 10.1053/j.gastro.2007.10.040]

76 **Chari ST**, Leibson CL, Rabe KG, Ransom J, de Andrade M, Petersen GM. Probability of pancreatic cancer following diabetes: a population-based study. *Gastroenterology* 2005; **129**: 504-511 [PMID: 16083707 DOI: 10.1016/j.gastro.2005.05.007]

77 **Ogawa Y**, Tanaka M, Inoue K, Yamaguchi K, Chijiiwa K, Mizumoto K, Tsutsu N, Nakamura Y. A prospective pancreatographic study of the prevalence of pancreatic carcinoma in patients with diabetes mellitus. *Cancer* 2002; **94**: 2344-2349 [PMID: 12015758 DOI: 10.1002/cncr.10493]

78 **Pannala R**, Basu A, Petersen GM, Chari ST. New-onset diabetes: a potential clue to the early diagnosis of pancreatic cancer. *Lancet Oncol* 2009; **10**: 88-95 [PMID: 19111249 DOI: 10.1016/S1470-2045(08)70337-1]

79 **Wang F**, Herrington M, Larsson J, Permert J. The relationship between diabetes and pancreatic cancer. *Mol Cancer* 2003; **2**: 4 [PMID: 12556242 DOI: 10.1186/1476-4598-2-4]

80 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]

81 **Arslan AA**, Helzlsouer KJ, Kooperberg C, Shu XO, Steplowski E, Bueno-de-Mesquita HB, Fuchs CS, Gross MD, Jacobs EJ, Lacroix AZ, Petersen GM, Stolzenberg-Solomon RZ, Zheng W, Albanes D, Amundadottir L, Bamlet WR, Barricarte A, Bingham SA, Boeing H, Boutron-Ruault MC, Buring JE, Chanock SJ, Clipp S, Gaziano JM, Giovannucci EL, Hankinson SE, Hartge P, Hoover RN, Hunter DJ, Hutchinson A, Jacobs KB, Kraft P, Lynch SM, Manjer J, Manson JE, McTiernan A, McWilliams RR, Mendelsohn JB, Michaud DS, Palli D, Rohan TE, Slimani N, Thomas G, Tjønneland A, Tobias GS, Trichopoulos D, Virtamo J, Wolpin BM, Yu K, Zeleniuch-Jacquotte A, Patel AV. Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Arch Intern Med* 2010; **170**: 791-802 [PMID: 20458087 DOI: 10.1001/archinternmed.2010.63]

82 **Li D**, Morris JS, Liu J, Hassan MM, Day RS, Bondy ML, Abbruzzese JL. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. *JAMA* 2009; **301**: 2553-2562 [PMID: 19549972 DOI: 10.1001/jama.2009.886]

83 **Calle EE**, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; **4**: 579-591 [PMID: 15286738 DOI: 10.1038/nrc1408]

84 **Rapp K**, Schroeder J, Klenk J, Stoehr S, Ulmer H, Concin H, Diem G, Oberaigner W, Weiland SK. Obesity and incidence of cancer: a large cohort study of over 145,000 adults in Austria. *Br J Cancer* 2005; **93**: 1062-1067 [PMID: 16234822 DOI: 10.1038/sj.bjc.6602819]

85 **Larsson SC**, Permert J, Håkansson N, Näslund I, Bergkvist L, Wolk A. Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. *Br J Cancer* 2005; **93**: 1310-1315 [PMID: 16288300 DOI: 10.1038/sj.bjc.6602868]

86 **Dawson DW**, Hertzer K, Moro A, Donald G, Chang HH, Go VL, Pandol SJ, Lugea A, Gukovskaya AS, Li G, Hines OJ, Rozengurt E, Eibl G. High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional KrasG12D mouse model. *Cancer Prev Res* (Phila) 2013; **6**: 1064-1073 [PMID: 23943783 DOI: 10.1158/1940-6207.CAPR-13-0065]

87 **Philip B**, Roland CL, Daniluk J, Liu Y, Chatterjee D, Gomez SB, Ji B, Huang H, Wang H, Fleming JB, Logsdon CD, Cruz-Monserrate Z. A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. *Gastroenterology* 2013; **145**: 1449-1458 [PMID: 23958541 DOI: 10.1053/j.gastro.2013.08.018]

88 **Oliver SG**. Functional genomics: lessons from yeast. *Philos Trans R Soc Lond B Biol Sci* 2002; **357**: 17-23 [PMID: 11839178 DOI: 10.1098/rstb.2001.1049]

89 **Garber K**. Energy boost: the Warburg effect returns in a new theory of cancer. *J Natl Cancer Inst* 2004; **96**: 1805-1806 [PMID: 15601632 DOI: 10.1093/jnci/96.24.1805]

90 **DeBerardinis RJ**, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 2008; **7**: 11-20 [PMID: 18177721 DOI: 10.1016/j.cmet.2007.10.002]

91 **Spratlin JL**, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. *Clin Cancer Res* 2009; **15**: 431-440 [PMID: 19147747 DOI: 10.1158/1078-0432.CCR-08-1059]

92 **Lai IL**, Chou CC, Lai PT, Fang CS, Shirley LA, Yan R, Mo X, Bloomston M, Kulp SK, Bekaii-Saab T, Chen CS. Targeting the Warburg effect with a novel glucose transporter inhibitor to overcome gemcitabine resistance in pancreatic cancer cells. *Carcinogenesis* 2014; **35**: 2203-2213 [PMID: 24879635 DOI: 10.1093/carcin/bgu124]

93 **WARBURG O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683 DOI: 10.1126/science.123.3191.309]

94 **Elstrom RL**, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM, Thompson CB. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 2004; **64**: 3892-3899 [PMID: 15172999 DOI: 10.1158/0008-5472.CAN-03-2904]

95 **Papandreou I**, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 2006; **3**: 187-197 [PMID: 16517406 DOI: 10.1016/j.cmet.2006.01.012]

96 **Kim JW**, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006; **3**: 177-185 [PMID: 16517405 DOI: 10.1016/j.cmet.2006.02.002]

97 **Lu CW**, Lin SC, Chen KF, Lai YY, Tsai SJ. Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J Biol Chem* 2008; **283**: 28106-28114 [PMID: 18718909 DOI: 10.1074/jbc.M803508200]

98 **Mathupala SP**, Heese C, Pedersen PL. Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumor suppressor p53. *J Biol Chem* 1997; **272**: 22776-22780 [PMID: 9278438 DOI: 10.1074/jbc.272.36.22776]

99 **Matoba S**, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM. p53 regulates mitochondrial respiration. *Science* 2006; **312**: 1650-1653 [PMID: 16728594 DOI: 10.1126/science.1126863]

100 **Bensaad K**, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 2006; **126**: 107-120 [PMID: 16839880 DOI: 10.1016/j.cell.2006.05.036]

101 **Kondoh H**, Lleonart ME, Gil J, Wang J, Degan P, Peters G, Martinez D, Carnero A, Beach D. Glycolytic enzymes can modulate cellular life span. *Cancer Res* 2005; **65**: 177-185 [PMID: 15665293]

102 **Britton D**, Zen Y, Quaglia A, Selzer S, Mitra V, Löβner C, Jung S, Böhm G, Schmid P, Prefot P, Hoehle C, Koncarevic S, Gee J, Nicholson R, Ward M, Castellano L, Stebbing J, Zucht HD, Sarker D, Heaton N, Pike I. Quantification of pancreatic cancer proteome and phosphorylome: indicates molecular events likely contributing to cancer and activity of drug targets. *PLoS One* 2014; **9**: e90948 [PMID: 24670416 DOI: 10.1371/journal.pone.0090948]

103 **Qin Y**, Zhu W, Xu W, Zhang B, Shi S, Ji S, Liu J, Long J, Liu C, Liu L, Xu J, Yu X. LSD1 sustains pancreatic cancer growth via maintaining HIF1α-dependent glycolytic process. *Cancer Lett* 2014; **347**: 225-232 [PMID: 24561118 DOI: 10.1016/j.canlet.2014.02.013]

104 **Chien W**, Lee DH, Zheng Y, Wuensche P, Alvarez R, Wen DL, Aribi AM, Thean SM, Doan NB, Said JW, Koeffler HP. Growth inhibition of pancreatic cancer cells by histone deacetylase inhibitor belinostat through suppression of multiple pathways including HIF, NFkB, and mTOR signaling in vitro and in vivo. *Mol Carcinog* 2014; **53**: 722-735 [PMID: 23475695 DOI: 10.1002/mc.22024]

105 **Kitamoto S**, Yokoyama S, Higashi M, Yamada N, Matsubara S, Takao S, Batra SK, Yonezawa S. Expression of MUC17 is regulated by HIF1α-mediated hypoxic responses and requires a methylation-free hypoxia responsible element in pancreatic cancer. *PLoS One* 2012; **7**: e44108 [PMID: 22970168 DOI: 10.1371/journal.pone.0044108]

106 **Selak MA**, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* 2005; **7**: 77-85 [PMID: 15652751 DOI: 10.1016/j.ccr.2004.11.022]

107 **Isaacs JS**, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J, Ratcliffe PJ, Linehan WM, Neckers L. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* 2005; **8**: 143-153 [PMID: 16098467 DOI: 10.1016/j.ccr.2005.06.017]

108 **King A**, Selak MA, Gottlieb E. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. *Oncogene* 2006; **25**: 4675-4682 [PMID: 16892081 DOI: 10.1038/sj.onc.1209594]

109 **Pollard PJ**, Brière JJ, Alam NA, Barwell J, Barclay E, Wortham NC, Hunt T, Mitchell M, Olpin S, Moat SJ, Hargreaves IP, Heales SJ, Chung YL, Griffiths JR, Dalgleish A, McGrath JA, Gleeson MJ, Hodgson SV, Poulsom R, Rustin P, Tomlinson IP. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. *Hum Mol Genet* 2005; **14**: 2231-2239 [PMID: 15987702 DOI: 10.1093/hmg/ddi227]

110 **MacKenzie ED**, Selak MA, Tennant DA, Payne LJ, Crosby S, Frederiksen CM, Watson DG, Gottlieb E. Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. *Mol Cell Biol* 2007; **27**: 3282-3289 [PMID: 17325041 DOI: 10.1128/MCB.01927-06]

111 **DeBerardinis RJ**, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci USA* 2007; **104**: 19345-19350 [PMID: 18032601 DOI: 10.1073/pnas.0709747104]

112 **Dang CV**, Kim JW, Gao P, Yustein J. The interplay between MYC and HIF in cancer. *Nat Rev Cancer* 2008; **8**: 51-56 [PMID: 18046334 DOI: 10.1038/nrc2274]

113 **Dang CV**. Rethinking the Warburg effect with Myc micromanaging glutamine metabolism. *Cancer Res* 2010; **70**: 859-862 [PMID: 20086171 DOI: 10.1158/0008-5472.CAN-09-3556]

114 **Christofk HR**, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL, Cantley LC. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 2008; **452**: 230-233 [PMID: 18337823 DOI: 10.1038/nature06734]

115 **Vander Heiden MG**, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029-1033 [PMID: 19460998 DOI: 10.1126/science.1160809]

116 **Jiang P**, Du W, Wang X, Mancuso A, Gao X, Wu M, Yang X. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. *Nat Cell Biol* 2011; **13**: 310-316 [PMID: 21336310 DOI: 10.1038/ncb2172]

117 **Denkert C**, Bucher E, Hilvo M, Salek R, Orešič M, Griffin J, Brockmöller S, Klauschen F, Loibl S, Barupal DK, Budczies J, Iljin K, Nekljudova V, Fiehn O. Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. *Genome Med* 2012; **4**: 37 [PMID: 22546809 DOI: 10.1186/gm336]

118 **Vermeersch KA**, Styczynski MP. Applications of metabolomics in cancer research. *J Carcinog* 2013; **12**: 9 [PMID: 23858297 DOI: 10.4103/1477-3163.113622]

119 **Zhou W**, Capello M, Fredolini C, Racanicchi L, Piemonti L, Liotta LA, Novelli F, Petricoin EF. Proteomic analysis reveals Warburg effect and anomalous metabolism of glutamine in pancreatic cancer cells. *J Proteome Res* 2012; **11**: 554-563 [PMID: 22050456 DOI: 10.1021/pr2009274]

120 **Urayama S**, Zou W, Brooks K, Tolstikov V. Comprehensive mass spectrometry based metabolic profiling of blood plasma reveals potent discriminatory classifiers of pancreatic cancer. *Rapid Commun Mass Spectrom* 2010; **24**: 613-620 [PMID: 20143319 DOI: 10.1002/rcm.4420]

121 **Bathe OF**, Shaykhutdinov R, Kopciuk K, Weljie AM, McKay A, Sutherland FR, Dixon E, Dunse N, Sotiropoulos D, Vogel HJ. Feasibility of identifying pancreatic cancer based on serum metabolomics. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 140-147 [PMID: 21098649 DOI: 10.1158/1055-9965.EPI-10-0712]

122 **Kobayashi T**, Nishiumi S, Ikeda A, Yoshie T, Sakai A, Matsubara A, Izumi Y, Tsumura H, Tsuda M, Nishisaki H, Hayashi N, Kawano S, Fujiwara Y, Minami H, Takenawa T, Azuma T, Yoshida M. A novel serum metabolomics-based diagnostic approach to pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 571-579 [PMID: 23542803 DOI: 10.1158/1055-9965.EPI-12-1033]

123 **Ritchie SA**, Akita H, Takemasa I, Eguchi H, Pastural E, Nagano H, Monden M, Doki Y, Mori M, Jin W, Sajobi TT, Jayasinghe D, Chitou B, Yamazaki Y, White T, Goodenowe DB. Metabolic system alterations in pancreatic cancer patient serum: potential for early detection. *BMC Cancer* 2013; **13**: 416 [PMID: 24024929 DOI: 10.1186/1471-2407-13-416]

124 **Zhang G**, He P, Tan H, Budhu A, Gaedcke J, Ghadimi BM, Ried T, Yfantis HG, Lee DH, Maitra A, Hanna N, Alexander HR, Hussain SP. Integration of metabolomics and transcriptomics revealed a fatty acid network exerting growth inhibitory effects in human pancreatic cancer. *Clin Cancer Res* 2013; **19**: 4983-4993 [PMID: 23918603 DOI: 10.1158/1078-0432.CCR-13-0209]

125 **Jain M**, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, Kafri R, Kirschner MW, Clish CB, Mootha VK. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* 2012; **336**: 1040-1044 [PMID: 22628656 DOI: 10.1126/science.1218595]

126 **ter Kuile BH**, Westerhoff HV. Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. *FEBS Lett* 2001; **500**: 169-171 [PMID: 11445079 DOI: 10.1016/S0014-5793(01)02613-8]

**P-Reviewer:** Falconi M, Gangl A, Hayano K **S-Editor:** Ma YJ **L-Editor: E-Editor:**

**Table 1 Pancreatic ductal adenocarcinoma related genetic syndromes**

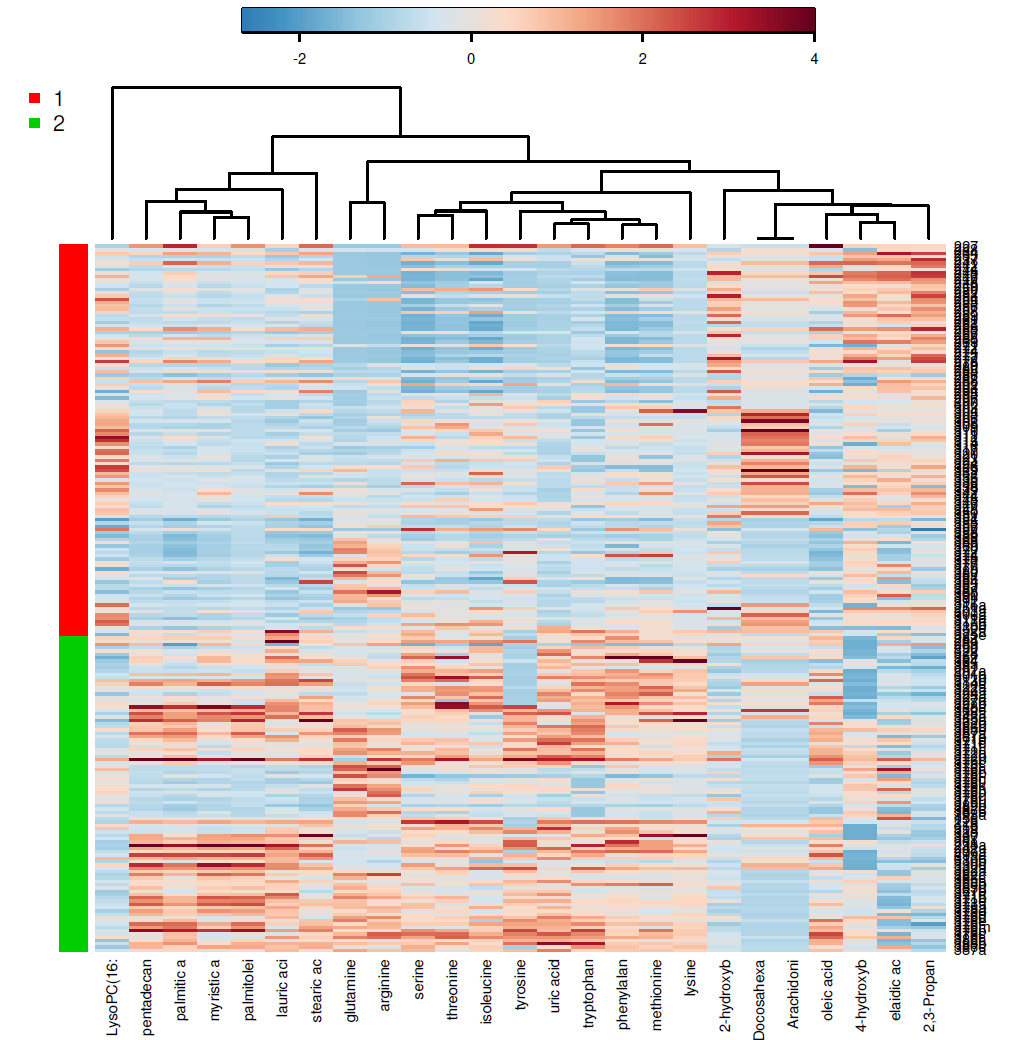
|  |  |  |  |
| --- | --- | --- | --- |
| Syndrome | Inheritance | Gene mutation | Risk of PDAC |
| Peutz-Jeghers syndrome[38] | Autosomal dominant (AD) | *STK11*/*LKB1* | Standardized incidence ratio (SIR) = 132 |
| Hereditary Pancreatitis[39-41] | AD | *PRSS1*  *SPINK1* | OR = 69.9 |
| Familial atypical multiple mole melanoma syndrome[42-44] | AD | *CDKN2A* | SIR = 13-38 |
| Hereditary breast-ovarian cancer syndrome[45-51] | AD | *BRCA2*  *BRCA1* | BRCA2: OR = 3.5-10-fold increased risk  BRCA1: OR = 2.26 times average population |
| Lynch syndrome[52] | AD | *MLH1*, *MSH2*, *MSH6* or *PMS2* | SIR = up to 8.6 |
| Cystic fibrosis[53] | Autosomal recessive | *CFTR* | OR = 5.3-6.6 |

PDAC: Pancreatic ductal adenocarcinoma.

**Table 2 Results of screening programs for pancreatic cancer in high-risk groups *n* (%)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study | CAPS1 | CAPS2 | CAPS3 | U of Washington | | FaPaCa | Dutch Study |
| Diagnostic Yield | 2 (5.3) | 8 (10) | 92 (43) | 10 (13) | 1 (1.3) | | 10 (23) |

Positive finding of abnormal imaging such as mass (solid, cyst) or abnormal duct in the cases. CAPS: Cancer of the Pancreas Screening Study; FaPaCa: Familial Pancreatic Cancer Study.



**Figure 1 example of metabolomics based analysis, allowing segregation of pancreatic ductal adenocarcinoma from benign cases.** Heat map illustration of discriminant capability of a metabolite set derived from gas chromatography and liquid chromatography/mass spectrometry plasma metabolomics dataset comparing pancreatic ductal adenocarcinoma patients (Red or group 1; *n =* 110) and benign pancreas (includes benign cysts, chronic pancreatitis, and normal pancreas) (Green or group 2; *n =* 90). Metabolites are plotted on x-axis, and the cases on the y-axis. Blue color indicates data points with a value smaller than the median of the respective metabolite and the red indicates higher values. This candidate set of metabolites enabled the segregation of the two groups.