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Differing molecular pathology of pancreatic adenocarcinoma in Egyptian and United States patients

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Variations in genetic mutations in pancreatic carcinoma between different populations have not been studied extensively, especially in developing countries where pancreatic cancer is rare. We studied the molecular pathology of 44 pancreatic carcinomas from patients residing in a heavily polluted region in the Nile River delta and compared the findings with tumors from 44 United States (US) patients. We evaluated *K-ras* mutations in codon 12, *p53* mutations in exons 5–8, and *Gadd45a* mutations in exons 1 and 4. Overall, rates of *K-ras*, *p53* and *Gadd45* mutations were not statistically different in tumors of patients from Egypt and the US (67.4 *vs.* 63.4%; 27.3 *vs.* 36.4% and 9.1 *vs.* 4.5%, respectively). However, there were distinct differences in the specific types of *K-ras* and *p53* mutations between the 2 groups. In *K-ras*, G → T transversion mutation was more frequent in the tumors from Egypt than from the US (58.6 *vs.* 26.9%), whereas G → C transversion was detected in 26.9% of US tumors but none from Egypt ($p = 0.003$). We also found a trend toward differences in the *p53* exons in which mutations occurred, with higher frequency of exon 5 mutation and lower frequency of exon 6 mutation in Egyptian tumors. Logistic regression showed that *K-ras* G → T transversion mutations and *p53* exon 6 mutations were predicted by the country of residence of the patients. Our study identifies that there are differences in the types of mutations found in tumors from pancreatic carcinoma patients in Egypt and the US, and suggests that environmental factors may explain these differences.

Key words: pancreatic cancer; molecular pathology; K-ras; p53; international differences

Studies of unusual cancer distribution and analysis of particular genetic mutations may provide clues to cancer etiology. We previously reported high incidence and mortality rates for colorectal carcinoma in young patients under age 40 in Egypt as compared to the United States.[1,2] We also reported patterns of genetic mutations in Egyptian colorectal cancers that were distinct from Western patients.[3] During our research on the epidemiology of colorectal cancer in Egypt, we observed an area in the northeast Nile River delta region with a high incidence of pancreatic cancer.[4] This region is known to have the highest environmental pollution of pesticides and heavy metals in Egypt.[5–9]

Pancreatic cancer incidence rates vary considerably throughout the world, with the highest rates occurring in developed countries.[10,11] This cancer shows a wide range of geographic incidence variation in the US with the Louisiana wetlands area and the Mississippi River delta regions reporting the highest rates.[12] Pancreatic cancer has not been widely reported in developing countries, except for the one area of high incidence we identified in the northeast Nile River delta region of Egypt.[4,13]

The molecular genetics of pancreatic carcinoma have been studied in detail. Mutations in the *K-ras* proto-oncogene and *p53* gene are common, and *Gadd45* mutations have been identified.[14] The study reported here was designed to evaluate the rates and types of these mutations in tumors from Egyptian patients who reside in the geographical region of extensive environmental pollution. The study also compared and contrasted the mutation characteristics with those of patients in the US.

Material and methods

This study included tumors obtained from surgically resected Stage II and III pancreatic cancer[15] patients in Egypt at the Gastrointestinal Surgery Center (GSC) of Mansoura University and in the US at the University of Texas, M. D. Anderson Cancer Center in Houston. The group from Egypt included 44 newly diagnosed patients with histopathologically confirmed ductal adenocarcinoma of the pancreas. The Egyptian cases represent all consecutive patients undergoing resection of the pancreas who had preliminary histopathological diagnosis of pancreatic adenocarcinoma and confirmation of the diagnosis in Houston by one of the authors (SRH). All Egyptian patients were residents of the Dakahleia Province where the GSC is located, and no age or gender restrictions were applied. The 44 Egyptian patients were all the patients who had surgical resections during the period of the study with recruitment from November 1998 to February 2000. These patients represented 26% of all incident pancreatic cancer patients seen at the study hospital during this period. This rate of histopatholgical confirmation in a developing country is lower than rates of histopathological confirmation in developed countries.[16]

The second group of tumors was obtained as archival tissue from the surgical pathology files of 44 selected US patients who underwent diagnosis and treatment for adenocarcinoma with surgical resection of the pancreas between 1992 and 2004 at The University of Texas, M. D. Anderson Cancer Center in Houston. Because of the young age of onset in Egyptian patients, the US cases were matched to the Egyptian patients as closely as possible by age (±10 years) and gender. Matching was successful for 40 pairs of patients, and 4 US patients were matched for age, but not

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for gender. Pancreatic cancer patients at M. D. Anderson Cancer Center represent the following 3 groups: (*i*) Patients diagnosed clinically and/or pathologically at the center (39% of patients), who therefore have pathologic specimens available for study; (*ii*) Patients diagnosed outside the center, who have subsequent clinical and/or pathological diagnosis confirmed at M. D. Anderson Cancer Center (52%) and therefore have pathologic specimens available; (*iii*) Patients diagnosed outside the center without tissue confirmation (9%). The 44 US patients were among the first 2 described groups. A list of all patients who fulfilled the age-matching criteria (*i.e.*, ±10 years) and gender was created.

The case–case design we used allows the comparison between case groups or across case subtypes. This design was first proposed by Begg and Zhang (1994)[17] to provide more efficient estimation of etiologic contrast parameters. It also allows the identification of factors that are differentially related to disease subtypes or different groups of cases from more than one population. Case–case odds ratio estimates highlight the relative strength of covariate-outcome associations between the 2 case groups.[18] Limitations of case–case analysis include lack of estimation of relative risk because of lack of control subjects. Case–case design has been used to explore differences in genetic mutational rates and types in different studies including ours.[3,19–21]

Ductal adenocarcinoma of the pancreas was confirmed by histopathological review (SRH). The Egyptian patients included in this study were participants in an ongoing case–control study to investigate the association between pancreatic cancer and cadmium exposure in Dakahleia, Egypt. Interviewers elicited epidemiologic information using a questionnaire that was used and tested in previous studies.[1,22,23] The questionnaire allowed collection of information about demographics, occupation (agricultural, professional and technical or administrative), residence (urban *vs.* rural), smoking and any family history of cancer. Interviews took place during hospital admission of patients for their preoperative investigations. Patients in Egypt are routinely admitted to hospitals for a period of 3–7 days before surgery for such preoperative clinical and laboratory evaluations. Trained interviewers who participated in our previous studies[3,23,24] conducted the interviews.

The medical records of the patients from M. D. Anderson Cancer Center were reviewed to retrieve the same data elements. A trained study coordinator at M. D. Anderson Cancer Center conducted the abstraction of medical records. The coordinator was supervised by 2 of the coauthors (ASS and MLB).

Because place of residence reported in the records of M. D. Anderson denoted only residence at the time of surgery, we could not determine the place of longest residence for these patients, and we did not use this variable in the data analysis of US patients. The study was approved by IRB committees in Egypt and the US.

Laboratory methods

Microdissection and DNA extraction. Areas of adenocarcinoma and non-neoplastic control tissue were microdissected from routine formalin-fixed, paraffin-embedded tissue sections cut 5-μm thick and stained with hematoxylin and eosin. DNA was extracted as in our previous studies.[25,26] Approximately one square centimeter of tumor tissue was wet with xylene, scraped from the slide using a clean razor blade and placed into a microcentrifuge tube. The xylene was removed by vacuum centrifugation until completely dry. Each specimen was then treated with 50 μl of buffer containing 0.5% Tween 20 (Boehringer Mannheim, Mannheim, Germany), 20 μg Proteinase K (Boehringer Mannheim, Mannheim, Germany), 50 mM Trizma Base at pH 8.9 and 2 mM EDTA. The samples were incubated at 56°C overnight. Proteinase K was inactivated by incubating the samples at 100°C for 10 min. A 1:20 dilution of the DNA lysate in nuclease-free water was used for PCR amplification. The extracted DNA was stored at −20°C until analysis.

K-ras *PCR amplification.* We evaluated codons 12 and 13 of the *K-ras* gene.[27] The mutations were classified for location in either the first or the second base. The 2 bases of *K-ras* were amplified in a 50 μl reaction volume using 2 μl of genomic DNA, 1× PCR Buffer II, 2 mM magnesium chloride, 0.8 mM dNTP mix, 2.25 U Ampli Taq™ Gold (Applied Biosystems, CA), 0.125 U Pfu DNA Polymerase (Stratagene, CA) and 20 pmol of each primer (forward primer, 5′-GGCCGGTAGTGTATTAACCTTATGTGTGACAT-3′, and reverse primer, 5′CCGCGGCCGGCGGCCAAAACAAGATTTACCTCTATTGTTGG-3′, Life Technologies, MD). PCR reactions were carried out using the following touchdown cycling conditions: denaturation at 95°C for 10 min; 14 cycles (95°C × 20 sec, 59°C × 60 sec [−0.5°C/cycle] and 72°C × 60 sec), 25 cycles (95°C × 20 sec, 52°C × 60 sec and 72°C × 60 sec); and extension at 72°C for 10 min. Thermal cycling was performed using a GeneAmp PCR System 9700 (Applied Biosystems, CA). The quality of the product was analyzed on a 6% polyacrylamide gel.

p53 *PCR amplification.* Exons 5 through 8 of the *p53* gene were amplified separately in 50 μl volumes using ~2 μl of the 1:20 diluted DNA lysate, 1× PCR Buffer II (Applied Biosystems, CA), an additional 100 nmol magnesium chloride, 40 nmol dNTP mix, 1.25 U Ampli Taq™ Gold (Applied Biosystems, CA) and 20 pmol of sense and antisense primer. The primer sequences were as follows:

Exon 5 (sense) 5′-GACTTTCAACTCTGTCTCC-3′, Exon 5 (antisense) 5′-GAGCAATCAGTGAGGAATC-3′, Exon 6 (sense) 5′-TCCCCAGGCCTCTGATTCC-3′, Exon 6 (antisense) 5′-TGACAACCACCCTTAACCC-3′, Exon 7 (sense) 5′-CAAGGCGCACTGGCCTCATC-3′, Exon 7 (antisense) 5′-CACAGCAGGCCAGTGTGCAG-3′, Exon 8 (sense) 5′-GATTTCCTTACTGCCTCTTGC-3′ and Exon 8 (antisense) 5′-GTGAATCTGAGGCATAACTGC-3′.

PCR amplification was carried out using the following cycling conditions: Exons 6 and 8, denaturation at 95°C for 10 min, 45 cycles (95°C × 60 sec, 61°C × 60 sec, 72°C × 60 sec), and extension at 72°C for 5 min; Exon 7, denaturation at 95°C for 10 min, 45 cycles (95°C × 60 sec, 65°C × 60 sec, 72°C × 60 sec), and extension at 72°C for 5 min; Exon 5 denaturation at 95°C for 10 min, 45 cycles (95°C × 60 sec, 55°C × 60 sec, 72°C × 60 sec), and extension at 72°C for 5 min. Thermal cycling was performed using a GeneAmp® PCR System 9700 (Applied Biosystems, CA). The quality of the product was examined and quantitated on a 2.0% agarose gel.

Gadd45 *PCR amplification.* The *GADD45a* gene fragment was amplified by PCR. Exon 1 and exon 4 were 265 and 397 bp, respectively. The PCR primers used were 5′-GCCTGTGAGTGAGTGCAGAA-3′ (sense) and 5′-GGAGTT GCCCTGTGCAAACT-3′ (antisense) for exon 1 and 5′-GAACCCAACTACCTTGAAGA-3′ (sense) and 5′-CCCCTTGGCATCAGTTTCTG-3′ (antisense) for exon 4. The sequencing primers were 5′-TAGCCGTGGCAGGAGCAG-3′ (sense) for exon 1 and 5′-TGTCTCCATGTCACATAGCC-3′ (sense) for exon 4. The PCR was run in 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 45 sec and extension at 72°C for 2 min with a Thermal cycler (Perkin-Elmer, Norwalk, CT).

Automated DNA Sequencing for K-ras *and* p53 *mutations.* PCR products were diluted to 5 ng/μl and purified by mixing 5 μl of PCR product dilution with 2 μl Exo/SAP (Amersham Life Science, OH), incubated at 37°C for 15 min, and then inactivated by incubating at 80°C for 15 min. DNA sequencing was performed in 20 μl volumes using 2 μl purified PCR product, 4 μl ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems, CA), and 10 pmol of forward primer using the following cycling conditions: initial denaturation at 95°C × 5 min followed by 25 cycles of 95°C × 20 sec, 52°C × 60 sec and 60°C × 60 sec. Following spin-column purification (Princeton Separations, NJ) and resuspension in 10 μl formamide, the reaction products were sequenced by capillary electrophoresis using an ABI PRISM™ 3700 or ABI 3730 DNA Analyzer (Applied Biosystems, CA). The sequences of samples containing mutations were confirmed using the reverse primer.

TABLE I – CHARACTERISTICS OF PANCREATIC ADENOCARCINOMA PATIENTS INCLUDED IN THE STUDY

	Egypt ($n = 44$)	United States ($n = 44$)	p-value
	N	*N*	
Age (years)			
Mean ± SD	52.70 ± 10.27	53.66 ± 10.35	0.665
Gender			
Male	25 (56.8)	29 (65.9)	
Female	19 (43.2)	15 (34.1)	0.381
Smoking**			
Yes	25 (56.8)	29 (72.5)	0.134
No	19 (43.2)	11 (27.5)	
Residence			
Rural	26 (59.1)	N/A	N/A
Urban	18 (40.9)	N/A	
Occupation			
Farming-related	12 (27.3)	3 (6.8)	0.011
Non-farming	32 (72.7)	41 (93.2)	
Family history of pancreatic cancer			
Yes	0 (0)	4 (9.1)	0.042
No	44 (100)	40 (90.9)	
Family history of any cancer			
Yes	2 (4.5)	22 (50.0)	<0.0001
No	42 (95.5)	22 (50.0)	
Genetic characteristics			
K-ras codon 12			
Mutation	29 (65.9)	26 (59.1)	0.698
Wild Type	14 (31.8)	15 (34.1)	
Unavailable	1 (2.3)	3 (6.8)	
p53 exons 5–8			
Mutation	12 (27.3)	16 (36.4)	0.360
Wild type	32 (72.7)	28 (63.6)	
Unavailable	0	0	
Gadd45 exons 1 and 4			
Mutation	4 (9.1)	2 (4.5)	0.693*
Wild Type	32 (72.7)	25 (56.8)	
Unavailable	7 (16)	17 (38.6)	0.017
K-ras and *p53*	8/29 (27.6)	11/26 (42.3)	0.447*
K-ras, *p53*, and *Gadd45*	2	0	N/A

Values in parentheses indicate percentage values.
*p of Fisher's exact test.
**Data unavailable for 4 US patients.

Gadd45 *sequence analysis.* We used the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Corp., Foster City, CA) for direct DNA sequencing. The PCR products were electrophoresed on a 2% agarose gel containing 0.5 mg/ml of ethidium bromide. The band of interest was excised and purified with a QIAquick Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany). A Microcon-100-Column (Millipore, Bedford, MA) was used to purify the PCR products without ectopic sites and confirmed by electrophoresis. For automated cycle sequencing, 20–40 ng of purified PCR product was subjected to sequencing PCR in a total volume of 20 μl of PCR reaction mixture containing 3.2 pmol of *p53* sequencing primer (sense) and 8 μl of BigDye Terminator Ready Reaction Mix (Applied Biosystems). The sequencing reaction was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer) for 20 cycles for exons 1 and 4. Each cycle consisted of denaturation at 96°C for 30 sec, annealing at 50°C for 15 sec and extension at 60°C for 4 min. Ethanol precipitation was used to purify the sequencing PCR products. After being denaturated at 95°C for 3 min, the purified sample was electrophoresed on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). Sequencing Analysis and Sequence Navigator Software (Applied Biosystems) were used to analyze the sequence data.

Statistical methods and analysis. Differences in frequencies between the Egyptian and US groups were evaluated by simple contingency table analysis (Fisher's exact probability test) using the SAS® statistical package (SAS®v8.2). Variables that were included in this analysis were age (used as a continuous variable), gender (male *vs.* female), smoking (yes *vs.* no), and residence for Egyptians (rural versus urban). Residence data for Egyptian patients reflected lifetime residence, which was not available for the US patients. Occupation was classified into 3 groups (agricultural, professional and technical or administrative), and family history of pancreatic cancer and family history of cancer in any relative were scored as yes *vs.* no. Total frequencies of *K-ras*, *p53* and *Gadd45* mutations were used in the analysis as categorical variables, using single nucleotide changes and position.

Unconditional logistic regression models were computed to test associations between mutations or mutation types and demographic, occupational and lifestyle factors. Contingency tables were constructed yielding chi-squared p-values, Fisher's exact p-values, crude odds ratio (OR) and 95% confidence interval (95% CI). Separate multiple logistic regression models were constructed for statistical analysis of *K-ras* G → T mutation and *p53* mutation in exon 6 as the dependent variables with other variables (*e.g.*, residence, gender, smoking and farming related occupations) as the covariates to test for confounding and effect modification.

TABLE II – DISTRIBUTION OF *K-RAS*, *P53*, AND *GADD45* MUTATION POSITION IN PANCREATIC ADENOCARCINOMAS OF PATIENTS FROM EGYPT AND THE UNITED STATES

	Exon	Egypt	United States	p-value
		N	*N*	
K-ras	Codon 12a	2 (6.9)	6 (23.08)	0.007
	Codon 12b	27 (93.1)	20 (76.9)	
p53	5	6 (21.4)	3 (10.7)	0.134*
	6	2 (16.7)	9 (56.3)	
	7	2 (16.7)	1 (6.3)	
	8	2 (16.7)	3 (18.8)	
Gadd45	1	0 (0)	0 (0)	N/A
	4	4 (100.0)	2 (100.0)	

Values in parentheses indicate percentage values.
*Fisher's exact test.

Results

Patient characteristics

The Egyptian patients were young (mean age of 52.7 ± 10.3 years) relative to the usual age of patients with pancreatic cancer in the US. Matching of cases for age was successful, as the mean age of the selected US patients was 53.7 ± 10.4 years ($p = 0.665$) (Table I). Males constituted 56.8 and 65.9% of the study sample from Egypt and the US, respectively, while females constituted 43.2 and 34.1%, respectively ($p = 0.381$) (Table I). There was no statistically significant difference between Egyptian and US patients regarding smoking ($p = 0.134$) (Table I). However, more Egyptian patients held farming related occupations (27.3%) than US patients (6.8%). Family history of cancer in any relative, and history of pancreatic cancers in relatives were significantly more frequent among US patients than among Egyptian patients: 50.0 and 4.5% for family history of cancer in US and Egyptian patients, respectively, and 9.1 and 0% for family history of pancreatic cancer in US and Egyptian patients, respectively (Table I).

Mutation frequencies

K-ras amplification and sequencing was successful in 43/44 (97.7%) of tumors from the US and in 41/44 (93.2%) of tumors from Egypt ($p = 0.3061$). *p53* amplification and sequencing was successful in all tumors. *Gadd45* amplification and analysis was successful in 37/44 (87.1%) of tumors from Egypt and 27/44 (61.4%) of tumors from the US ($p = 0.0167$) (Table I).

Overall, the rates of *K-ras*, *p53* and *Gadd45* mutations were not statistically different in tumors of patients from Egypt and the US (65.9 *vs.* 59.1%, 27.3 *vs.* 36.4%, and 9.1 *vs.* 4.5%, respectively) (Table II). Over one-quarter (27.6%) of Egyptian patients and 28.6% of US patients had both *K-ras* and *p53* mutations ($p = 0.946$) (Table II).

Tumors from 2 Egyptian patients, but none of the tumors from the US, had concurrent mutations in all 3 genes (*K-ras*, *p53* and

TABLE III – RELATIONSHIP BETWEEN SPECIFIC *K-ras* MUTATIONS AND PATIENT CHARACTERISTICS

	Egypt (n = 29)	United States (n = 26)	p-value
	N	*N*	
K-ras			
Codon 12			
G → T	17 (58.6)	7 (26.9)	0.003[1]
G → A	12 (41.4)	12 (46.2)	
G → C	0 (0)	7 (26.9)	
G → T (17,7)**			
Age			
Mean ± SD	53.29 ± 10.71	53.71 ± 10.96	0.933
Gender			
Male	8 (47.1)	4 (57.1)	0.500*
Female	9 (52.9)	3 (42.9)	
Smoking			
Yes	7 (41.2)	6 (100.0)	0.017*
No	10 (58.8)	0 (0)	
Residence			
Rural	12 (70.6)	0 (0)	N/A
Urban	5 (29.4)	0 (0)	
Occupation			
Farmers	4 (23.5)	1 (14.3)	1.00
Nonfarmers	13 (76.5)	6 (85.7)	
G → A (12,12)**			
Age (years)			
Mean ± SD	52.83 ± 10.77	58.25 ± 8.01	0.176
Gender			
Male	10 (83.3)	8 (66.7)	0.923*
Female	2 (16.7)	4 (33.3)	
Smoking			
Yes	11 (91.7)	9 (75.00)	0.953*
No	1 (8.3)	3 (25.00)	
Residence			
Rural	7 (58.3)	0 (0)	N/A
Urban	5 (41.7)	0 (0)	
Occupation			
Farmers	6 (50.0)	0 (0)	0.014
Non Farmers	6 (50.0)	12 (100)	

Values in parentheses indicate percentage values.
[1]Two-sided p from Fisher's exact test.
*Right-sided p from Fisher's exact test.
**Values in parentheses indicate number of tumors from Egypt and US, respectively.

Gadd45). In tumors from US patients with a family history of pancreatic cancer, 1 patient had a *K-ras* 12b G → A mutation, and another had a 12b G → T mutation. One patient had both a *p53* (exon 6 T → G) and *K-ras* mutation (12b G → T).

Types of mutations

The rates of individual mutations of *K-ras* and *p53* were different between the 2 groups of tumors. *K-ras* mutations showed a higher rate of mutation in codon 12a in tumors from the US than in tumors from Egypt (23.1 *vs.* 6.9%, $p = 0.007$) (Table II). We also found that the 2 groups of tumors had specific *K-ras* mutations with different frequencies (Fisher's exact $p = 0.003$) (Table III). The frequency of G → A transitions was similar between Egyptian and US tumors at 41.4 and 46.2%, respectively, with the most noteworthy difference observed in G → T and G → C transversions. *K-ras* G → T transversions appeared in 58.6% of the Egyptian tumors and in 26.9% of the US tumors. The *K-ras* G → C transversions appeared in none of the Egyptian tumors and in 26.9% of the US tumors (Table III).

There was also a trend toward different individual types of *p53* mutations, although the differences were not statistically significant. About one-fifth (21.4%) of *p53* mutations in Egyptian tumors were in exon 5 *vs.* 10.7% of tumors from the US. On the contrary, *p53* exon 6 mutations were more frequent in tumors from the US 56.3 *vs.* 16.7% in tumors from Egypt. Among tumors with *p53* mutations, exon 7 mutations were somewhat more frequent in tumors from Egypt than in tumors from the US (16.7 *vs.* 6.3%).

TABLE IV – LOGISTIC REGRESSION MODEL TO PREDICT G → T TRANSVERSIONS IN *K-ras* IN PANCREATIC ADENOCARCINOMAS FROM EGYPT AND THE UNITED STATES

	OR (95% CI)	p-value*
Unadjusted Country		
United States	1	
Egypt	3.85 (1.23–12.01)	0.020
Adjusted Country		
United States	1	
Egypt	4.78 (1.22–18.67)	0.049
Sex		
Male	2.43 (0.69–8.59)	0.169
Female	1	
Smoking		
Yes	1.83 (0.49–6.81)	0.365
No	1	
Farming-Related Occupation		
Yes	0.58(0.12–2.82)	0.503
No	1	

Exon 8 mutations in tumors from Egypt and the US were present in 16.7 *vs.*18.8%, in Egypt and the U.S., respectively (Table II). Although comparison of all types of *p53* mutations in tumors from Egypt and the US was not statistically significant ($p = 0.134$) (Table II), pancreatic cancers from the US were more likely to have mutations in *p53* exon 6 when compared to the frequency of mutations in exons 5, 7 and 8 combined ($p = 0.044$) (Table V). *Gadd45* mutations were detected only in exon 4, and 9% of tumors from Egypt had *Gadd45* mutations compared with 4.5% of tumors from the US (Table II).

We found that the 2 groups of tumors had specific *K-ras* mutations with different frequencies (Fisher's exact $p = 0.003$) (Table III). The frequency of G → A transitions was similar between Egyptian and US tumors at 41.4 and 46.2%, respectively, with the most noteworthy difference observed in G → T and G → C transversions. *K-ras* G → T transversions appeared in 58.6% of the Egyptian tumors and in 26.9% of the US tumors. The *K-ras* G → C transversions appeared in none of the Egyptian tumors and in 26.9% of the US tumors (Table III).

Association of mutations and patient characteristics

All tumors from the US with *K-ras* G → T transversions were from smokers (100%) (1 missing smoking data), while 41.2% of tumors from Egyptian patients with this mutation were smokers ($p = 0.017$). Half the Egyptian tumors with *K-ras* G → A transitions were from farmers, while none of the US tumors with this mutation were from farmers (Fishers exact $p = 0.014$) (Table III). No statistically significant differences were observed in occupation or smoking of patients whose tumors had G → T transversions from Egypt and the US. Additionally, we found that among the Egyptian patients there was no association with having a *K-ras* G → T transversion or a mutation in p53 exon 6 and living in a rural *vs.* urban area ($p = 0.495$ and $p = 0.9500$, respectively) (data not shown).

Logistic regression analysis showed that country of residence of patients (Egypt *vs.* US) was an important predictor of *K-ras* G → T mutations ($p = 0.020$) (Table IV).

After adjusting for gender, smoking and occupation as potential confounders, country of residence was strongly and independently predictive of *K-ras* G → T transversion ($p = 0.049$), and no evidence of confounding was identified when tested against other confounders (Table IV). We found that tumors from Egypt were 3.85 times more likely to have this mutation than others (95% CI: 1.23–12.01).

Country of residence of patients was also an important predictor of *p53* exon 6 mutations in models that adjusted for gender, smoking and occupation (Table V). Odds ratios of country of residence ranged from OR = 6.4 (CI: 0.8–204.9) ($p = 0.044$) for the unadjusted model to OR = 24.2 (CI: 1.5–393.4) ($p = 0.025$) for the partially adjusted model that included gender and occupation. The

TABLE V – LOGISTIC REGRESSION MODEL TO PREDICT *p53* EXON 6 MUTATIONS IN PANCREATIC ADENOCARCINOMAS FROM EGYPT AND THE UNITED STATES

	OR (95% CI)	*p*-value*
Unadjusted		
Country		
Egypt	1	
United States	6.43 (1.05–39.33)	0.044
Model 1		
Country		
Egypt	1	
United States	12.92 (0.82–204.94)	0.068
Sex		
Female	1	
Male	3.19 (0.28–36.71)	0.352
Smoking		
No	1	
Yes	0.30 (0.03–2.66)	0.280
Farming-related occupation		
No	1	
Yes	4.55(0.23–89.91)	0.320
Model 2		
Country		
Egypt	1	
United States	24.24 (1.49–393.38)	0.025
Sex		
Female	1	
Male	3.43 (0.31–38.33)	0.317
Farming-related occupation		
No	1	
Yes	5.54 (0.31–97.58)	0.243
Model 3		
Country		
Egypt	1	
United States	8.72 (0.77–99.12)	0.081
Sex		
Female	1	
Male	3.72 (0.31–44.4)	0.298
Smoking		
No	1	
Yes	0.26(0.03–2.16)	0.211

fully adjusted model that included gender, smoking and occupation showed an OR = 12.9 (CI: 0.8–204.9) ($p = 0.068$). The model that included gender and smoking only showed OR = 8.7 (CI: 0.8–99.1) ($p = 0.081$). We observed that smoking did not add significantly to the model and that the final model should be model 2 (Table V). However, we think that the 3-fold change in point estimate between models 2 and 3 along with a 2-fold change in the point estimate between models 1 and 2 reflect the impact of geographical differences between mutation pattern and other covariates in addition to country of residence.

Discussion

Although the rates of mutations in the *K-ras*, *p53* and *Gadd45* genes in the 2 groups of pancreatic adenocarcinomas included in this study did not show a statistically significant difference, the individual types of mutations showed distinct differences between patients from Egypt and the US. The overall rates of *K-ras*, *p53* and *Gadd45* mutations observed in this study for tumors from Egypt and the US were comparable to other previous studies.[28–33]

The prevalence of *K-ras* mutations in pancreatic cancer has been reported in many studies with a wide range of frequencies. A recent meta-analysis of the studies reporting on *K-ras* and pancreatic intraepithelial neoplasia (PanIN) published in peer-reviewed journals from 1988 to 2003 showed a stepwise increase in *K-ras* mutations with the grade of dysplasia of the PanIN lesions. *K-ras* mutations were found in 36, 44 and 87% of PanIN-1a, 1b and 2–3 lesions, respectively (trend statistic $p < 0.001$).[34] Previous studies comparing the prevalence of *K-ras* mutations in tumors from African-American and Caucasian pancreatic cancer patients in Detroit showed *K-ras* mutations in 70% of African Americans and 73% of Caucasians.[35] Other studies showed *K-ras* mutations in 62, 71 and 75% in subjects from Japan,[36] China[37] and Austria,[28] respectively.

p53 has been reported with rates between 50 and 75% in different studies.[38] Rates similar to ours have been reported in other studies, *i.e.*, 38[36] and 41%.[32]

Only 4 tumors (11.1%) from Egyptian patients and 2 tumors (6.5%) from US patients had *Gadd45* mutations, all in exon 4. *Gadd45* mutations are uncommon in pancreatic cancer, and the rate observed in this study in Egyptian tumors was similar to that found in a previous study in Japan.[14] However, the rate of *Gadd45* mutations observed in tumors from US patients in this study was lower than that reported in the study from Japan.[14] Interpopulation genetic differences resulting from selection of young US patients for matching may be a reason for the differences in mutational rates from previous studies.

The rates of specific mutations in *K-ras* and *p53* in the tumors from the 2 countries showed distinct differences in our study. *K-ras* G → T mutations were significantly more frequent in tumors from Egyptian patients than in US patients, and G → C mutations were identified in tumors from US patients, but not in any tumor from Egyptian patients. Furthermore, exon 5 mutations in *p53* were more frequent in tumors from Egyptian patients, while exon 6 mutations were more frequent in tumors from US patients. Rates of types of *K-ras* and *p53* mutations in tumors from US patients in this study were comparable to previous studies from western countries.[39] The distinct mutational types in this study (*K-ras* G → T mutations and p53 exon 6 mutations) were predicted by the country of patient's residence (Egypt or the US), after adjusting for age, gender, smoking and occupation.

Our study also found that a high proportion of Egyptian patients were farmers and rural residents, with presumed exposure to organochlorine pesticides.[1,22] Those compounds have been associated with *K-ras* G → T mutations in pancreatic cancer mutations in a previous study in Spain.[40] Other prevalent environmental exposures in our Egyptian study locale include heavy metals, such as cadmium and chromium.[5–9] Our recent study in this region in Egypt revealed significantly higher serum cadmium levels in pancreatic cancer patients than in controls.[24] Using industrial assessment, and the Finnish job-exposure matrix, Alguacil *et al.*[20] analyzed the occupational exposures of 107 incident pancreatic cancer patients from Spain. This study reported that the association between chromium and *K-ras* mutations was statistically significant for G → T transversions. Hence, organochlorine pesticides and heavy metals may have contributed to the types of mutations seen in tumors from Egyptian patients at significantly higher rates than tumors from the US.

About one-quarter (24.2%) of tumors from Egyptian patients and 36.7% of tumors from US patients included in this study had both *K-ras* and *p53* mutations, which was similar to previous studies.[32,41,42] Blanck *et al.*[42] reported 64% of pancreatic cancer patients with both *K-ras* and *p53* mutations had the same type of mutation (transition or transversion), which suggested an environmental agent might have acted on both genes in a similar manner, or that the particular DNA base is prone to mutation.[42]

Subjects included in this study had high rates of cigarette smoking with higher rates in the US patients than in Egyptians. Smoking has been linked to pancreatic cancer in many studies and has been the most consistent risk factor for pancreatic cancer. However, smoking was not related to specific *K-ras* or *p53* mutations in this study.

Few studies have focused on subjects with a family history of pancreatic cancer. A case report described the characteristics of a mucinous pancreatic duct hyperplasia with a strong family history of pancreatic carcinoma.[43] This reported patient had *K-ras* mutations, with 5 of the 7 duct lesions harboring activating point mutations in codon 12 of *K-ras*. Four were G → A transitions, and a fifth was a G → C transversion. In contrast, these lesions did not harbor any mutations in exons 5–8 of the *p53* gene, nor was there overexpression of the p53 protein as determined by immunohistochemistry.

This finding indicated that *K-ras* mutation is an early event.[43] All the subjects in our study had Stage II or III disease and exhibited the same results: 3 of 4 had *K-ras* mutations, but only 1 was detected with *p53* mutations, which also suggests that *K-ras* mutations occur earlier than *p53* mutations. Another study reported a possible relationship of family history of cancer to the expression of *p53* in pancreatic tumors.[44] A lower incidence of *p53* expression observed in patients with a family history of cancer suggests normal expression of *p53* protein is present in a majority of patients who develop pancreatic tumors related to other inherited or familial risk factors.[44]

Our study has strengths that support the validity of the findings. First, the histopathological diagnosis and confirmation of pancreatic cancer diagnoses by a single pathologist is an advantage. Second, the comparability of age, gender and tumor stage through our case matching method minimized the bias of comparison between tumors included in the study. Third, the heavily polluted locale from which patients were recruited in Egypt was an ideal place to examine the role of intense environmental exposures on pancreatic carcinogenesis. However, the lack of information about the ethnicity of US patients limited our ability to make inferences about the relationship between mutational types and ethnicity among US patients. For example, African-Americans have higher risk for pancreatic cancer than whites.[45] Another limitation of our study was the eliciting of family history of cancer for US patients from the medical records with its possible recall bias and misclassification.

There might have been some incomplete comparability between the 2 sources of lifestyle factors' information. However, medical records at M. D. Anderson are comprehensive and several ongoing projects aim at eliciting detailed lifestyle and behavioral factors.

It is interesting to note that the results of this study did not show lower *K-ras* mutation rates in tumors from Egyptian patients compared with tumors from US patients, as shown in our previous study comparing colorectal cancers from the 2 countries (11% in tumors from Egypt *vs.* 67% in tumors from the US).[3] However, the types of mutations were significantly different in the pancreatic tumors from the 2 countries. The *K-ras* gene may play a different role in the molecular pathway in different cancers. *K-ras* mutation is an early event in pancreatic cancer,[46] and the majority of pancreatic cancer tumors should have *K-ras* mutations, but the time at which the mutation occurs may be induced by different environmental exposures.[40,47–50]

In summary, pancreatic cancers from Egyptian patients in this study had a unique pattern of *K-ras* and *p53* mutations that was significantly different from the patterns of mutations detected in tumors from US patients. The differences in *K-ras* and *p53* mutations may be associated with specific environmental exposures, such as organochlorine pesticides or heavy metals, especially in the heavily polluted region where the Egyptian patients were recruited.[24] Future studies should compare the variety of mutations for patients in different regions in Egypt and the US with different pollution levels. Such studies may provide more evidence to support the association between mutational types and environmental exposures. Determining the level of cadmium and other heavy metals in blood or tissue of pancreatic cancer patients, and examining their association with mutational types, may provide clues about the etiology of pancreatic cancer and the role of gene–environmental interactions in pancreatic carcinogenesis.

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