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Differences in Molecular Pathologic Characteristics of Pancreatic Adenocarcinoma between Egyptian and Moroccan Patients

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Abstract

Introduction: Pancreatic cancer has not been well studied, especially in developing countries.

Materials and Methods: We studied the variations in genetic mutations in pancreatic adenocarcinoma between Moroccan and Egyptian populations. The molecular pathology of 30 tumors from a large hospital in Casablanca, Morocco were examined and compared with the findings of 44 tumors from the Gharbiah Governate in Egypt. K-ras mutations in codons12 and 13 in addition to p53 mutations in exons 5-8 were evaluated.

Results: Overall, differences in the rates of K-ras mutations were not statistically significant (48.00 and 34.09%, respectively); however differences in rates of p53 mutations were statistically significant with p53 mutations more common in Moroccan tumors than in Egyptian tumors (46.67 and 16.28%, respectively). G→T mutations of the K-ras gene were most commonly seen Egyptian tumors, whereas G→A mutations were the most common type of mutations in Moroccan tumors. Logistic regression analysis showed that a p53 mutation in any exon as well as a p53 mutation in exon 5 predicted the country of residence and those mutations occurred more frequently in Moroccan patients.

Conclusion: Our study shows that differences exist within the Arab population in the molecular pathology of both the K-ras and p53 genes. Further studies are necessary to clarify the differences in molecular pathways of pancreatic cancer in the Middle East and to investigate the role of environmental and/or genetic factors related to those pathways.

Keywords: Pancreatic cancer, Molecular pathology, K-ras, p53, Egypt, Morocco

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Introduction

Pancreatic cancer is a rare but deadly disease. Unfortunately, little is known about the etiology of this disease. The strongly recognized risk factors are age and smoking, however suspected risk factors include: diabetes, chronic pancreatitis, and environmental or occupational exposures.[1] This disease accounts for approximately 2% of all cancers worldwide; however the one year survival rate is only 16% and five year survival is around 5% making it the most fatal of all major cancers.[2]

Countries participating in the Middle East Cancer Consortium (MECC) have reported variations in the incidence of this disease.[3] In Jordan, the incidence of pancreatic cancer was only 1.1% from 1996 to 2001 whereas in Egypt and Cyprus, these numbers have risen to 1.9% of total cancers. Turkey also has a moderate incidence rate, but exact figures were not available for this time period. Israel has the highest proportion of pancreatic cancer in MECC countries, with rates comprising 2.5% of all cancers.[3] In Morocco, for the year 2004, the Ibn Roch'd Cancer registry has shown that pancreatic cancer comprised only 1.5% of all cancers. Other areas of North Africa have also reported a low incidence of pancreatic cancer.[4]

p53 mutations are the most common human mutations in cancer.[5] K-ras mutations in codon 12 are the most common mutations in pancreatic cancer and can be considered as a "signature" of pancreatic cancer.[6] Previous studies have shown differences in genetic mutations in K-ras and p53 genes between Egyptian pancreatic tumors and pancreatic tumors from the United States.[7] The overall mutation rates are not statistically significant, but there are distinct differences in the specific types of K-ras and p53 mutations between tumors of patients from those countries. Both the K-ras G→T transversion mutation and p53 exon 6 mutations predict the country of residence (Egypt) of the patients.[6]

Our group also investigated the relationship between environmental pollution and pancreatic cancer mutations within Egypt. Our study showed higher rates of K-ras G→T mutations in the polluted Mansoura region versus less polluted non-Mansoura regions.[8] The total mutation rates were higher for both K-ras and p53 mutations in polluted regions which might suggest a link between pollution and pancreatic cancer mutations.[7]

Previous studies also show different histopathological patterns amongst colorectal tumors in Egypt, Turkey and Jordan.[9] Tumor histopathology differed among the three countries with Jordan having the highest rate of methylation patterns in p-16 genes and CIMP-high genes.[9] In this study, we investigated K-ras and p53 mutations in two countries in the Middle East, Egypt with a moderate prevalence for pancreatic cancer and Morocco, a country with a low prevalence for the disease. The aim of the study was to explore if the difference in prevalence of pancreatic cancer between the two countries was associated with a difference in K-ras and p53 mutation rates and types of pancreatic cancers.

Materials and Methods

This study included tumors collected from patients who underwent surgical resection in the Gharbiah Governate in Egypt or at the Ibn Roch'd Hospital in Casablanca, Morocco. The group from Egypt was identified through the Gharbiah Cancer Registry. All cases of confirmed primary pancreatic adenocarcinoma with paraffin embedded tumor tissues were included regardless of age or gender. Cases were identified from the years 2000 - 2006. The Egyptian group consisted of 32 cases that underwent surgical resection at one of several centers within the governate. Added to the 32 cases were 12 cases from a previous study on Egyptian patients who also lived in the Gharbiah Governate or were treated at the Tanta Cancer Center.[8] All cases were confirmed by histopathological analysis by Egyptian pathologists and verified by one of the co-authors (JFG).

The group from Morocco consisted of cases retrospectively identified from the pathology records of Ibn Roch'd Hospital. All cases of confirmed primary pancreatic adenocarcinoma with paraffin

embedded tumor tissues were included with no restrictions on age, gender, or residency. Cases were identified from the years 2003-2007. The Moroccan group consisted of 30 cases that underwent surgical resection at Ibn Roch'd Hospital. All cases were confirmed by histopathological analysis by Moroccan pathologists, and verified in the same manner as the Egyptian tumors.

In both locations, demographic information such as: age, gender, residency, tumor stage, tumor grade, smoking status, diabetes and occupation were collected.

This study was approved by Institutional Review Boards in Egypt, Morocco and the University of Michigan.

Laboratory analysis

Microdissection and DNA extraction

Areas of adenocarcinoma and non-neoplastic control tissue were microdissected from routine formalin-fixed, paraffin-embedded tissue sections that were cut 3-µm thick and stained with hematoxylin and eosin. DNA was extracted by scraping tumor tissue with a clean razor blade and placing the tissue into a micro centrifuge tube. Control tissue was scraped in the same manner. For de-paraffinization, 500 µl xylene was added. Xylene was removed by centrifugation at 13,000 rpm and the supernatant was subsequently removed by pipetting. Afterwards, 100% ethanol (500 µl) was added and removed in the same manner; this step was repeated twice. Tubes were heated at 37º C for 30 minutes until all ethanol was evaporated and the remaining pellet was resuspended in 180 µl of ATL solution to which 20 µl of proteinase K was added. The tubes were then incubated in a water bath at 56 ºC overnight (for a minimum of 3 hours). AL buffer (200 µl) was added and the tubes were incubated in a water bath at 70º C for 10 minutes. Ethanol (200 µl) on ice was added and the tubes were allowed to remain at room temperature for 5 minutes. The mixture was centrifuged briefly and the entire contents were applied to a QIAamp Spin Column, which was then centrifuged at 6000 g for 1 minute. After centrifuging, the column was then placed in a clean 2 ml collection tube and 500 µl of AW1 was added. The tubes were centrifuged again at 6000 g for 1 minute and the column was again placed in a clean 2 ml collection tube. AW2 (500 µl) was added and the tubes were centrifuged at full speed for 5 minutes. After the solution was discarded, the tubes were re-centrifuged at full speed for 2 minutes. The column was placed in a new clean collection tube, 80 µl of AE was added, and the tubes were incubated in a water bath at 70º C for 5 minutes. After incubation the tubes were centrifuged at 6000 g for 1 minute. The column was placed in a clean 1.5 ml tube where the entire 100 µl of product was reapplied to the column and then centrifuged at 6000 g for 1 minute. The extracted DNA was stored at -20º C until analysis.

K-ras PCR amplification

We evaluated codons 12 and 13 of the K-ras gene. The two codons were amplified in a 50 µl reaction volume using 30.5 µl distilled water, 2 µl genomic DNA, 5 µl 10x, 4 µl magnesium chloride, 4 µl of dNTP mix (2.5mM) and 0.5 µl Ampli TaqTM Gold (Applied Biosystems, USA) as well as 2 µl of each primer (forward primer, 5' GGCCTGCTGAAAATGACTGAA - 3', and reverse primer, 5' CAAAGAATGGTCCTGCACCA GTAA - 3', (Integrated DNA Technologies, Inc., USA). PCR reactions were carried out using the following touchdown cycling conditions: denaturation at 95ºC for 10 minutes; 14 cycles at 95ºC for 320 seconds, at 59ºC for 360 seconds [20.5ºC/cycle] and at 72ºC for 360 seconds, 25 cycles at 95ºC for 320 seconds, at 52ºC for 360 seconds and at 72ºC for 360 seconds; and extension at 72 ºC for 10 minutes. Thermal cycling was performed using either a Peltier Thermal Cycler PTC-100 or a Peltier Thermal Cycler PTC-200 (MJ Research, USA). The quality of the product was analyzed on a 2% agarose gel.

p53 IHC staining

Paraffin sections were cut on a microtome and heated for 20 minutes at 65 ºC. Slides were deparaffinized in xylene, 3 changes of two minutes

each and then rehydrated with graduated alcohol (two minutes each), ending with distilled water (100% alcohol, 95% alcohol, 70% alcohol, and water). Citrate buffer, pH 6.0, was microwaved for 10 minutes with a 10 minute cooling time and a 15 minute running water wash. Slides were placed in a peroxidase block for 5 minutes followed by a buffer rinse. The primary p53 antibody (Dako Cytomation, Denmark) was added in an approximate 1:50 dilution and left for 30 minutes at room temperature, followed by a buffer rinse. EnVision+Labeled Polymer (mouse; Dako Cytomation, Denmark) was added to the slides and left for 30 minutes, followed by a buffer rinse. DAB Chromagen was then added and left for 5 minutes, followed by a water rinse. Slides were subsequently dehydrated using graduated alcohol with two minutes per step (70%, 95%, 100% alcohol). Slides were finally dipped in xylene with 3 changes, two minutes each, and covered with cover slips.

p53 PCR amplification

Exons 5 through 8 of the p53 gene were amplified separately in 50 µl volumes using 29.7 µl distilled water, 2 µl genomic DNA, 5 µl 10x, 4 µl magnesium chloride, 5 µl of dNTP mix (2.5 mM), 0.3 µl Ampli TaqTM Gold (Applied Biosystems, USA) and 2 µl of sense and antisense primer. The primer sequences were as follows:

Exon 5 (sense) 5'- TTCACTTGTGCCCT-GACTTTCAA -3', Exon 5 (antisense) 5'-GTGAGCAGCTGGGGCTGG -3' (Integrated DNA Technologies, Inc., USA)

Exon 6 (sense) 5'- CCCAGGCCTC TGATTCCTCA -3', Exon 6 (antisense) 5'-GGAGGGCCACTGACAACCAC - 3' (Integrated DNA Technologies, Inc., USA)

Exon 7 (sense) 5'- AAAAAGGCCTC CCCTGCTTG - 3', Exon 7 (antisense) 5'- AGT-GTGCAGGGTGGCAAGTG - 3' (Integrated DNA Technologies, Inc., USA)

Exon 8 (sense) 5'- GGGACAGGTAGGAC-CTGATTTCC - 3' and Exon 8 (antisense) 5'-CCACCGCTTCTTGTCCTGCT -3' (Integrated DNA Technologies, Inc., USA)

PCR amplification was carried out using the following cycling conditions: exons 6 and 8, denaturation at 95 ºC for 10 minutes, 45 cycles (95 ºC for 60 seconds, 61 ºC for 60 seconds, 72 ºC for 60 seconds), and extension at 72 ºC for 5 minutes; exon 7, denaturation at 95 ºC for 10 minutes, 45 cycles (95 ºC for 60 seconds, 65 ºC for 60 seconds, 72 ºC for 60 seconds), and extension at 72 ºC for 5 minutes; exon 5 denaturation at 95 ºC for 10 minutes, 45 cycles (95 ºC for 60 seconds, 55 ºC for 60 seconds, 72 ºC for 60 seconds), and extension at 72 ºC for 5 minutes. Thermal cycling was performed using either a Peltier Thermal Cycler PTC-100 or a Peltier Thermal Cycler PTC-200 (MJ Research, USA). The quality of the product was examined and quantitated on a 2.0% agarose gel.

Automated DNA sequencing for K-ras and p53 mutations

PCR products were purified by mixing 10 µl PCR product dilution with 4 µl Exo/SAP (USB Corporation, USA), incubated at 37ºC for 15 minutes, and inactivated by incubating at 80ºC for 15 minutes. DNA sequencing was performed in 20 µl volumes using 2µl purified PCR product, 4 µl ABI PRISM1 BigDyeTM Terminator Cycle Sequencing Kit (Applied Biosystems, USA), and 10 pmol of forward primer using the following cycling conditions: initial denaturation at 95ºC for 35 min followed by 25 cycles of 95ºC for 20 seconds, 52ºC for 60 seconds and 60ºC for 60 seconds. Following spin-column purification (Princeton Separations, USA) and resuspension in 10 µl formamide, the reaction products were sequenced by capillary electrophoresis using an ABI PRISMTM 3700 or ABI 3730 DNA Analyzer (Applied Biosystems, USA). The sequences of samples containing mutations were confirmed using a reverse primer.

Statistical methods and analysis

Differences in frequencies between the Moroccan and Egyptian groups were evaluated with chi-square analysis and Fisher's exact test when cell counts were smaller than 5 using the SAS® statistical package (SAS® v 9.1).Variables included in this analysis were age (continuous

variable), gender, smoking (yes vs. no), residence (urban vs. rural), diabetes (yes vs. no), employment (yes vs. no) and tumor stage (1, 2, 3 or 4). Total frequencies of K-ras and p53 mutations were used in the analysis as categorical variables as well as specific mutations (yes vs. no).

Unconditional logistic regression models were used to test associations between rate and mutational types and demographic and tumor characteristics. Multiple logistic regression models were constructed for statistical analysis of mutations in any p53 exon and mutations in p53 exon 5 as the dependent variables with other variables such as sex, stage, smoking status, and residency as the covariates to test for confounding and effect modifications.

Results

Patient characteristics

The mean age of Egyptian patients was less than the Moroccans, but this difference was not statistically significant. Males constituted 43.33% and 68.18% of the study sample from Morocco and Egypt, while females constituted 56.67% and 31.25%, respectively, which was statistically significant (P= 0.0334). Moroccan patients were statistically less likely to be smokers than Egyptian patients (39.29 and 63.64%, respectively; P=0.0432). Moroccan patients were also less likely to be employed than Egyptian patients (38.46 and 65.91%, respectively), which was statistically significant (P= 0.0255). There were no statistically significant differences between Moroccan and Egyptian patients regarding area of residence (P= 0.1142), incidence of diabetes (P= 0.1947) or tumor grade (P= 0.1381). Table 1 summarizes all of the above data.

Mutation frequencies

Immunohistochemistry (IHC) staining results were available for 30/30 (100%) tumors from Morocco and for 32/44 (72.73%) of the tumors from Egypt. More than half (53.33%) of the tumors from Morocco stained positive with IHC and 68.75% of those from Egypt were positive, which was not statistically significant (P= 0.2130).

K-ras amplification and sequencing was successful in 25/30 (83.33%) of tumors from Morocco and in 44/44 (100%) of tumors from Egypt. p53 amplification and sequencing were successful in at least one of the four exons in 30/30 (100%) of tumors from Morocco and in 43/44 (97.73%) of tumors from Egypt.

Overall, the rates of K-ras mutations were not statistically different in tumors from Morocco and Egypt (48.00% vs. 34.09%), respectively (P= 0.2552). However, the rates of mutations in p53 genes differed considerably between Morocco and Egypt (46.67% vs. 16.28%, respectively); with Morocco having a significantly higher number of mutations (P< 0.0048).

There were 19 Moroccan patients (73.04%) with a mutation in either the K-ras or p53 gene. In contrast, 20 patients (45.45%) from Egypt had mutations in either gene, which was statistically significant (P= 0.0246). Mutations in both the K-ras and p53 genes (36.84%) were seen in more than 25% of the tumors from Moroccan patients. In contrast, only 10.00% of tumors from Egyptian patients had mutations in both the K-ras and p53 genes. This difference was statistically significant (P= 0.0255). Table 1 summarizes all of the above data.

Types of mutations

The rates of individual mutations of K-ras and p53 were different between the two groups of tumors. There were two cases from Egypt which had mutations in codon 13 of the K-ras genes while no patients from Morocco had mutations in codon 13. Differences in codon 12 mutations were present, as 60.00% of the Egyptians tumors showed G→T mutations while only 8.33% of Moroccan tumors had G→T mutations (P= 0.0814; Table 2). Of the K-ras mutations in Moroccan tumors, 91.67% were G→A, whereas 40.00% of the Egyptian tumors had G→A mutations which was statistically significant (P= 0.0082) (Table 2).

p53 mutations were most common in exon 5 in Egyptian tumors with a mutation to wild type ratio of 0.11, while p53 mutations were most common in exon 6 of the Moroccan tumors with a mutation to wild type ratio of 0.50 (Table 3). An

Table 1. Characteristics of pancreatic adenocarcinoma patients from Morocco and Egypt.

	Morocco (n=30)	Egypt (n=44)	*P*-value
Age (years)			
Mean +/- SD	60.50 +/- 12.63	55.63 +/- 10.02	0.0695*
Gender			
Female	17 (56.67)	14 (31.25)	
Male	13 (43.33)	30 (68.18)	0.0334
Smoking			
Yes	11 (39.29)	28 (63.64)	
No	17 (60.71)	16 (36.36)	0.0432
Residence			
Rural	5 (17.24)	15 (34.09)	
Urban	24 (82.76)	29 (65.91)	0.1142
Diabetes			
Yes	5 (19.23)	12 (34.29)	
No	21 (80.77)	23 (65.71)	0.1947
Work			
Yes	10 (38.46)	29 (65.91)	
No	16 (61.54)	15 (34.09)	0.0255
Tumor Characteristics			
Tumor Grade			
1	10 (38.46)	7 (21.88)	
2	13 (50.00)	20 (62.50)	
3	3 (11.54)	3 (9.38)	
4	0 (0)	2 (6.25)	
Unavailable	4	12	0.1381
IHC Staining for p53			
None	14 (46.67)	10 (31.25)	
Mild	5 (16.67)	6 (18.75)	
Moderate	5 (16.67)	6 (18.75)	
Marked	6 (20.00)	10 (31.25)	
**Any staining	16 (53.33)	22 (68.75)	0.2130
Genetic Characteristics			
K-ras codons 12 and 13			
Mutation	12 (48.00)	15 (34.09)	
Wild-type	13 (52.00)	29 (65.91)	
Unavailable	5	0	0.2552
P53 exons 5-8			
Mutation	14 (46.67)	7 (16.28)	
Wild-type	16 (53.33)	36 (83.72)	
Unavailable	0	1	0.0048
K-ras or p53	19 (73.04)	20 (45.45)	0.0246
K-ras and p53	7/19 (36.84)	2/20 (10.00)	0.0255 **

**P* (t-test)

***P* (Fisher's exact test)

Table 2. Relationship between specific K-ras codon mutations and patient characteristics.

	Morocco (n=12) (%)	Egypt (n=15) (%)	*P* value
K-ras			
G → T	1 (8.33)	9 (60.00)	0.0814*
G → A	11 (91.67)	6 (40.00)	0.0082*
G →T (1,9) **			
Age			
Mean +/- SD	68.00	53.00+/- 8.38	0.1280***
Gender			
Male	1 (100.0)	6 (66.67)	
Female	0 (0)	3 (33.33)	1.000*
Smoking			
Yes	1 (100.00)	6 (66.67)	
No	0 (0)	3 (33.33)	1.000*
Residence			
Rural	0 (0)	2 (22.22)	
Urban	1 (100)	7 (77.78)	1.000*
Diabetes			
Yes	0 (0)	0 (0)	
No	1 (100)	6 (100)	Unable to calculate
Work			
Yes	0 (0)	6 (66.67)	
No	0 (0)	3 (33.33)	Unable to calculate
G →A (11,6) **			
Age			
Mean +/- SD	59.55 +/- 10.91	55.50 +/- 15.57	0.5383***
Gender			
Male	5 (45.45)	4 (66.67)	
Female	6 (54.55)	2 (33.33)	0.6199*
Smoking			
Yes	5 (45.45)	4 (66.67)	
No	6 (54.55)	2 (33.33)	0.6199*
Residence			
Rural	2 (18.18)	1 (16.67)	
Urban	9 (81.82)	5 (83.33)	1.000*
Diabetes			
Yes	2 (18.18)	3 (60.00)	
No	9 (81.82)	2 (40.00)	0.2445*
Work			
Yes	5 (50.00)	5 (83.33)	
No	5 (50.00)	1 (16.67)	0.3069*

*Fisher's exact test
**Values in parentheses indicate number of tumors from Morocco and Egypt, respectively.
***t-test *P* value

interesting finding is that tumors in three Moroccans and one Egyptian had mutations in two p53 exons.

Association of mutations with patient characteristics

Analyzing specific K-ras mutations with patient characteristics showed no statistically significant differences. There were no associations between gender, smoking status, or residence in G→T transversions (*P*= 1.000, 1.000 and 1.000, respectively). We were unable to calculate *P*-values for diabetes or employment in G→T transversions. These results must be interpreted with caution as only one Moroccan tumor had a G→T transversion. There were no associations

Table 3. Distribution of K-ras mutation in codon 12 or 13 and of p53 mutation in exons 5 - 8.

	Morocco No. of patients	Ratio of Mutant to Wild-type	Egypt No. of patients	Ratio of Mutant to Wild-type	*P* value
Type of K-ras mutation					
G to T	1	0.08	9	0.31	0.2536
G to A	11	0.85	6	0.21	0.0219
G to C	0	0	0	0	N/A
Wild-type	13		29		
Type of p53 mutation					
Exon 5	4	0.25	4	0.11	0.4218
Exon 6	8	0.50	2	0.06	0.0097
Exon 7	3	0.19	1	0.03	0.1082
Exon 8	2	0.13	1	0.03	0.2469
Wild-type	16		36		

*Number of mutations in each specific exon reflects three cases in Morocco that had mutations in two exons, and one case in Egypt that had mutations in two exons.
*All *P* values are calculated using Fisher's exact test.

between gender, smoking status, place of residence, diabetes, or employment in G→A transversions (*P*= 0.6199, 0.6199, 1.000, 0.2455, 0.3069, respectively) (Table 2).

Logistic regression analysis showed that patients' country of residence was an important predictor of p53 mutations (*P*=<0.0001) (Table 4). After adjusting for gender and tumor stage (the two variables with the most information) as potential confounders, country of residence was still independently predictive of p53 mutations (*P*=0.0110). Logistic regression also showed that country of residence was an important predictor of having a p53 exon 5 mutation (*P*=0.0004). After adjusting for gender and tumor stage the effect still existed, though the association was not as strong (*P*=0.0627).

Discussion

Although the rates of mutations in K-ras genes did not differ between Moroccan and Egyptian patients, the type of mutations did. G→A mutations were the most common mutation in Moroccan cases, while Egyptian cases showed both G→T and G→A mutations, with G→T being slightly more common. The rates of K-ras mutations found in this study were much lower than in previous studies. A previous study involving Egyptian patients found K-ras mutations in 65.9% of tumors while this study only saw mutations in 34.1% of tumors.[6] Other studies have shown rates in patients as follows: US (79%), Chinese (76%), Japanese (91%), Austrian (75%), Dutch (93%), English (78%), Italian (73%) and Spanish (78%).[10] However, another study in Egypt which looked at the differences in rates between areas of residence (Mansoura and non-Mansoura) found significant differences between the two. The rates of K-ras mutations in this study were quite similar to the non-Mansoura cases (34.1 and 34.2%, respectively).[8] It might be that this difference in mutation rates was due to Egyptian patients who resided in less polluted areas.

The rates of mutations in the p53 genes differed between Moroccan and Egyptian patients in this study. p53 mutations were much more common in Morocco than in Egypt. In comparison to a previous study, the mutation rates in Morocco were similar to those seen in the United States (46.67 and 36.4%, respectively),[8] which suggested that rates in Morocco were closer to rates in the United States than Egypt. The rate of p53 mutations in Egyptian cases was lower than the previous study which compared Egypt to the United States (16.28 and 27.3%, respectively), but greater than the rate seen in non-Mansoura tumors in a second study (11.6%).[7, 8] This data has suggested that the rates seen in our study were mostly similar to the non-Mansoura regions of Egypt, which was logical because the Gharbiah governorate would be located in the non-Mansoura region, a less polluted area.

Table 4. Logistic regression model to predict p53 mutations, specifically mutations in exon 5 in pancreatic adenocarcinomas from Morocco and Egypt.

	OR (95% CI)	*P* value
Total p53 mutations (any exon)		
Country (unadjusted)		
Morocco	1	
Egypt	0.222 (0.075, 0.655)	0.0048
Country (Adjusted)*		
Morocco	1	
Egypt	0.094 (0.0015, 0.573)	0.0222
Gender		
Female	1	
Male	6.963 (0.670, 72.385)	0.1043
p53 mutations (exon 5)		
Country (unadjusted)		
Morocco	1	
Egypt	0.667 (0.153, 2.905)	0.5901
Country (Adjusted)*		
Morocco	1	0.8900
Egypt		
Gender		
Female	1	
Male	4.023 (0.372, 43.517)	0.2519

* Adjusted for gender and tumor stage.

The specific mutation rates in K-ras and p53 in the tumors from the two countries showed distinct differences in our study. Egyptian cases were more likely to have K-ras G→T mutations while Moroccan cases were more likely to have G→A mutations. Moroccan patients were more likely to have mutations in the p53 gene, specifically in exon 6. Country of residence was predictive of mutations in the p53 gene, particularly exon 5.

This current study has several strengths and many limitations. To our knowledge, this is the first study to investigate pancreatic cancer mutations in Morocco. Studies have been completed looking at Egyptian groups, but this is the first comparative study of pancreatic cancer mutations in North Africa or Middle Eastern countries.

One of the limitations of this study is the sample size. Pancreatic cancer is rare and has a low prevalence in the countries involved in this study. Although all possible cases have been included, the sample size is still small. Another limitation is the generalizability of the results. Every attempt has been made to define the population, however without complete demographic information, it is hard to be certain that all cases fit within the defined population. Thus, we must be cautious when generalizing the results.

Other useful studies would include looking at various types of pancreatic cancer besides adenocarcinoma. Ductal cancer and ampullary cancer are two types of pancreatic cancers that have different molecular characteristics with different consequences on survival and prognosis. In general, ampullary cancers are diagnosed earlier, have a better prognosis and also have common mutations in K-ras and p53 genes.[11-17]

This study shows that there are differences even within similar populations. The reasons for these differences are still unknown, but environmental factors may be an important consideration. These two distinct populations within North Africa share many similarities, both in lifestyle and within the molecular pathology of tumors. With increasing smoking rates, adaptation of a western lifestyle, increasing obesity and pollution in the Middle East, future studies should focus on environmental and lifestyle factors in

relation to both pancreatic cancer mutations and pathways in the Middle East.

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References

1. Kriegel AM, Soliman AS, Zhang Q, El Ghawalby N, Ezzat F, Soultan A, et al. Serum cadmium levels in pancreatic cancer patients from the east Nile delta region of Egypt. *Environ Health Perspect* 2006;114(1):113-9.
2. The American Cancer Society. Cancer Facts and Figures, 2009. Available from: http://www.cancer.org/docroot/STT/STT-O.asp.
3. Freedman LS, Edwards BK, Ries LAG, Young JL (eds). Cancer incidence in four member countries (Cyprus, Egypt, Israel, and Jordan) of the Middle East Cancer Consortium (MECC) compared with US SEER. *National Cancer Institute*, 2006. NIH Pub. No. 06-5873. Bethesda. Available from: http://seer.cancer.gov/publications/mecc/.
4. Registre des cancers Region Du Grand Casablanca. *Rapport de l'Annee* 2004.
5. Saif W, Karapanagiotou L, Syrigos K. Genetic alterations in pancreatic cancer. *World J Gastroenterol* 2007;13(33):4423-30.
6. Talar-Wojnarowski R, Malecka-Panas E. Molecular pathogenesis of pancreatic adenocarcinoma: Potential clinical implications. *Med Sci Monit* 2006;12(9): 186-93.
7. Soliman AS, Bondy M, Webb CR, Schottenfeld D, Bonner J, El-Ghawalby N, et al. Differing molecular pathology of pancreatic adenocarcinoma in Egyptian and United States patients. *Int J Cancer* 2006;119(6):1455-61.
8. Soliman AS, Lo A C, Banerjee M, El-Ghawalby N, Khaled HM, Bayoumi S, et al. Differences in K-ras and p53 gene mutations among pancreatic adenocarcinomas associated with regional environmental pollution. *Carcinogenesis* 2007;28(8):1794-9.
9. Chan AO, Soliman AS, Zhang Q, Rashid A, Bedeir A, Houlihan PS, et al. Differing DNA methylation patterns and gene mutation frequencies in colorectal carcinomas from Middle Eastern countries. *Clin Cancer Res* 2005;11(23):8281-87.
10. Dong M, Nio Y, Tamura K, Song M, Guo KJ, Guo RX, et al. Ki-ras point mutation and p53 expression in human pancreatic cancer: A comparative study among Chinese, Japanese, and Western patients. *Cancer Epidemiol Biomarkers Prev* 2000;9(3):279-84.
11. Suwa H, Ohshio G, Okada N, Wang Z, Fukumoto M, Imamura T, et al. Clinical significance of serum p53 antigen in patients with pancreatic carcinomas. *Gut* 1997;40(5):647-53.
12. Koopmann J, Fedarko NS, Jains A, Maitra A, Iacobuzio-Donahue C, Rahman A, et al. Evaluation of osteopontin as biomarker for pancreatic adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2004;13(3):487-91.
13. Bel Hadj N, Elloumi H, Babba T, Kchaou-Oukaa A, Gargouri D, Kochlef A, et al. Carcinoma of the papilla of vater. Diagnostic and therapeutic problems. An analysis of 32 Tunisian cases. *Tunis Med* 2006;84(11):701-4.
14. Huibregtse K, Offerhaus GJA, Slebos RJC. Clinical significance of K-ras oncogene activation in ampullary neoplasms. *Clin Pathol* 1996;49(6):460-64.
15. Barauskas G, Gulbinas A, Pundzius J. Results of surgical treatment of carcinoma of papilla of Vater. *Medicina (Kaunas)* 2007;43(6):455-62.
16. Want LF, Hingorani SR, Tuveson DA. Detecting and diagnosing ampullary neoplasms. *Cancer Biol Ther* 2004;3(7):657-59.
17. Van Heek NT, Maitra A, Koopmann J, Fedarko N, Jain A, Rahman A, et al. Gene expression profiling identifies markers of ampullary adenocarcinoma. *Cancer Biol Ther* 2004;3(7):651-56.