The role of gene-environment interaction in the development of pancreatic cancer

Tobacco smoking, family history of cancer and DNA repair genotypes as risk factors for pancreatic ductal adenocarcinoma: A molecular epidemiological case-control study

Rajiv Lochan Jayadeva

Thesis submitted towards MD examination

Institute of Cellular Medicine, Newcastle University

and

Department of Surgery, Freeman Hospital

Resubmission May 2013

Abstract:

The aetiology for sporadic pancreatic adenocarcinoma is poorly characterized. Familial/hereditary causes account for about 10% cases and tobacco smoking is a well-established risk, however it is only responsible for about a third of cases. DNA repair mechanisms restore the genome damage caused by carcinogens including those derived from tobacco smoking. Increasing attention is being focused on single nucleotide polymorphisms, which exist amongst various physiological pathways including DNA repair mechanisms, and account for inter-individual variation in risk for cancer. This study is an effort to investigate the impact of family history of malignancy, tobacco smoking and selected genetic polymorphisms involved in DNA repair on pancreatic ductal adenocarcinoma development.

A hospital-based case-control study of pancreatic adenocarcinoma cases and hospital-based controls was undertaken at the Freeman Hospital between 2005-2006. Pancreatic cancer cases were ascertained based on histology, cytology or a combination of clinical findings, tumour marker levels and progressive radiological changes. All participants were interviewed to establish a detailed clinical, family history and tobacco smoking (MONICA questionnaire) A sample of peripheral blood was obtained for genotyping of specific Base Excision repair genotypes – hOGG1, XRCC194, XRCC280, XRCC399 and APE148. Statistical analysis was performed on SPSS v16. Odds ratios (95% CI) were calculated for individual variables.

Tobacco smoking was confirmed to be a risk factor for pancreatic cancer [OR (95% CI) on univariate: ever smoker [(present and past smokers) OR 3.01 (95% CI 1.73 to 5.24)] and multivariate analysis: present smokers [OR = 8.531 (3.198 to 22.759) and past smokers OR = 5.862 (2.223 to 15.460)]. Importantly a significantly decreased cumulative tobacco exposure was seen amongst pancreatic cancer cases with a family

history of cancer [mean (SD): 30.00 (24.77) pack-years] as compared those who did not have such a history [44.69 (28.47) pack-years p=0/023]. No specific overall increased risk was associated with the individual base excision repair genotypes on both uni-variate and multi-variate analysis.

Tobacco smoking is a risk factor and appears to play a more important role (for pancreatic cancer development) in the presence of a family history of cancer. Small risks associated with SNP's are difficult to tease out from small studies like the current one. Larger multi institutional studies (as have been achieved for e.g. Lung Cancer) are required to confirm this latter finding and perform pooled analysis of data for specific sequence variants within a target biochemical pathway to uncover the risks associated with these genes.

Acknowledgment

Guru Brahma Guru Vishnu Guru Devo Maheshwara,
Guru Sakshat Param Brahma Tasmai Sri Guruve Namaha*

"To the teacher who is creation, to the teacher who is this very life, to the teacher who is all challenge and transformation, to the teacher within each of us, to the teacher beyond all things – formless and divine, I bow down and offer my life and all my efforts".

I would like to acknowledge my patients who have been the source of my entire learning and especially to those who have kindly volunteered to be a part of the current study notwithstanding their ill health

I remain grateful to my mentors Mr R.M Charnley and Prof A.K. Daly for their guidance and support in the conception, development, execution, data analysis and interpretation of the findings of this study

I am indebted to my family for all their support over the past years

Lastly but most importantly I am most indebted to my parents, who have been my first and constant teachers.

**In Sanskrit by Adi Sankaracharya (the foremost of ancient Indian teachers)*

in "Gurustotram" (hymns to the teacher)

Abbreviations

AJCC	American Joint Committee on Cancer
APE	Apurinic/apyrimidinic endonuclease
ATM	Ataxia telangiectasia mutated
APC	Adenomatous polyposis coli
BER	Base Excision Repair
BRCA	Breast cancer antigen
bp	base pair
CA-19-9	Carbohydrate antigen 19-9
CEA	Carcinoembryonic antigen
CBD	Common Bile Duct
CFTR	Cystic fibrosis transmembrane regulator
CF	Cystic Fibrosis
CNS	Central nervous system
CAD	Coronary artery disease
CVA	Cerebrovascular disease
COPD	Chronic obstructive pulmonary disease
DPC4	Deleted in pancreas cancer 4
DSB	Double strand break
DNA	Deoxyribonucleic acid
ERCP	Endoscopic retrograde cholangio-pancreatography
ESPAC	European study group on pancreatic cancer
EGFR	Epidermal growth factor receptor

EUS-FNA	Endoscopic ultrasound scan – Fine needle aspiration
EDTA	Ethylenediaminetetraacetic acid
FPC	Familial pancreatic cancer
FAP	Familial adenomatous polyposis
FAMMM	Familial atypical multiple mole melanoma
FEN1	Flap Endonuclease 1
FDR	First Degree relatives
FHP	Family history positive
FHN	Family history negative
FRH	Freeman Hospital
HW equilibrium	Hardy Weinberg equilibrium
hOGG1	Human 8-oxoguanine DNA glycosylase 1
HPB	Hepato-Pancreato-Biliary
Her2	Human epidermal growth factor receptor
HR	Homologous recombination
HNPCC	Hereditary non-polyposis colorectal cancer
HCl	Hydrochloric acid
IPMN	Intra-ductal mucinous neoplasm
IAP	International association of pancreatology
Kras	Kirsten rat sarcoma oncogene (homolog)
LREC	Local Research Ethics Committee
MgCl2	Magnesium Chloride
MSI	Microsatellite instability
MMR	Mis-match repair

MONICA	Monitoring of Cardio-vascular risks questionnaire
MCN	Mucinous cystic neoplasm
MDCT	Multi-detector computed tomography
MRI	Magnetic resonance Imaging
MRCP	Magnetic resonance cholangiopancreatography
NTP	Nucleotide Tri-phosphate
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNN	N′-nitrosonornicotine
NAT	N′-nitroso- anatabine
NAB	N′-nitrosoanabasine
NER	Nucleotide Excision Repair
NHEJ	Non-homologous end joining
PVD	Peripheral vascular disease
PAH	Polycyclic aromatic hydrocarbons
PanIn	Pancreatic intraepithelial neoplasia
pol β	Polymerase β
OR (95% CI)	Odds ratio (95% Confidence interval)
PDAC	Pancreatic ductal adenocarcinoma
PJS	Peutz Jeghers Syndrome
PD	Pancreatic duct
PV	Portal Vein
PARP	Poly ADP-ribose polymerase

PCR	Polymerase chain reaction
rpm	rotations per minute
RFLP	Restriction fragment length polymorphism
SEER	Surveillance epidemiology and End results
SCN	Serous cystic neoplasm
SNP	Single nucleotide polymorphisms
SD	Standard deviation
TSNA	Tobacco specific nitrosamines
TCR	Transcription coupled repair
TNM staging	Tumour Node Metastases staging
UV	Ultra-violet
WHO	World Health Organization
XRCC1	X-Ray Repair Cross-Complimenting1 Group
XPC	Xeroderma pigmentosum complementation group C
XPD	Xeroderma pigmentosum complementation group D

TABLE OF CONTENTS

ABSTRACT: 2
ABBREVIATIONS 5
LIST OF TABLES AND FIGURES 13

CHAPTER 1: INTRODUCTION 17
SECTION 1.1: PANCREATIC CANCER - EPIDEMIOLOGY 18
SECTION 1.2: PANCREATIC CANCER - CLINICAL FEATURES, MANAGEMENT AND OUTCOME 21
1.2.1 Clinical Features 21
1.2.2 Assessment, Diagnosis and Staging: 22
1.2.3 Management and outcome 25
1.2.3.1 Biliary decompression: 25
1.2.3.2 Resectable pancreas cancer: 26
1.2.3.3 Loco-regional disease: 29
1.2.3.4 Metastatic disease: 30
SECTION 1.3: MAJOR RISK FACTORS IN THE DEVELOPMENT OF PANCREATIC CANCER 33
1.3.1 Heritable causes and Familial pancreatic cancer 33
1.3.1.1 Hereditary Pancreatitis: 36
1.3.1.2 Cystic Fibrosis: 36
1.3.1.3 Peutz-Jeghers Syndrome (PJS) 37
1.3.1.4 Familial atypical multiple mole melanoma (FAMMM): 38
1.3.1.5 Hereditary breast and ovarian cancer (HBOC) 38
1.3.1.6 Lynch Syndrome (Hereditary non-polyposis colorectal cancer HNPCC) 39
1.3.1.7 Familial adenomatous polyposis (FAP) 39
1.3.1.8 Familial pancreatic cancer (FPC): 40
1.3.2 Age: 41
1.3.3 Race: 42
1.3.4 Gender: 43
1.3.5 Tobacco smoking: 43
1.3.5.1 Tobacco derived carcinogens 46
1.3.5.2 Tobacco derived carcinogens and pancreatic tumours in animals: 47
1.3.5.3 Tobacco carcinogens in humans and their relevance to pancreatic cancer: 49
1.3.5.4 Epidemiological studies: 50
1.3.6 Environmental factors including industrial exposure to chemicals 51
SECTION 1.4: PANCREATIC CARCINOGENESIS 52
1.4.1 Molecular progression model 52
1.4.1.1 PanIN-1A (Pancreatic Intraepithelial Neoplasia 1-A) 53
1.4.1.2 PanIN-1B (Pancreatic Intraepithelial Neoplasia 1-B) 53
1.4.1.3 PanIN-2 (Pancreatic Intraepithelial Neoplasia 2) 53
1.4.1.4 PanIN-3: (Pancreatic Intraepithelial Neoplasia 3) 53
1.4.2 Neoplastic pancreatic cysts: 55
1.4.2.1 Mucinous cystic neoplasms: 57
1.4.2.2 Intraductal papillary mucinous neoplasms (IPMN): 58
SECTION 1.5: DNA REPAIR 61
1.5.1 Base excision repair (BER): 61
1.5.2 Nucleotide Excision Repair (NER): 65
1.5.3 Double Strand Break Repair (DSB): 66
1.5.4 Mis-Match Reapir (MMR): 67
1.5.5 Diseases caused by inherited defects in DNA repair genes 69
1.5.6 Cancer due to DNA repair gene mutations 71

SECTION 1.6: INTER-INDIVIDUAL VARIATION IN RISK FOR CARCINOGENESIS 72
1.6.1 BER gene variants and their role in development of malignancy 74
1.6.1.1 hOGG1 74
1.6.1.2 XRCC1 Arg194Trp 76
1.6.1.3 XRCC1 Arg280His 76
1.6.1.4 XRCC1 Arg399Gln 76
1.6.1.5 Summary 77
1.6.2 DNA repair, pancreatic adenocarcinoma & need for further studies 79

CHAPTER 2: RESEARCH STUDY AND METHODS 82

SECTION 2.1 AIMS 83
SECTION 2.2 METHODS 84
2.2.1 Recruitment of cases 85
2.2.2 Questionnaire on Tobacco smoking and Family history of cancer 86
2.2.3 Collection of peripheral blood 87
2.2.4 Storage of samples 87
2.2.5 Preparation of DNA from peripheral blood 87
2.2.6 Genotyping protocols 88
2.2.6.1 Polymerase Chain Reaction methodology 88
2.2.6.2 Primers 89
2.2.6.3 Restriction fragment length polymorphism analysis 89
2.2.7 Power calculation 95
2.2.8 Statistical analysis 97

CHAPTER 3: RESULTS - INFLUENCE OF A CUMULATIVE TOBACCO EXPOSURE AND FAMILY HISTORY OF CANCER IN INDEX CASES OF PANCREATIC CANCER 98
SECTION 3.1 INTRODUCTION 99
SECTION 3.2 CASES AND CONTROLS 103
3.2.1 Gender 103
3.2.2 Age: 103
3.2.3 Smoking status: 104
3.2.4 Family history: 105
SECTION 3.3 TOBACCO EXPOSURE AND RISK OF PANCREATIC CANCER 108
SECTION 3.4 FAMILY HISTORY OF CANCER IN CAFDRS 112
SECTION 3.5 INTERACTION BETWEEN TOBACCO SMOKING AND FAMILY HISTORY OF CANCER IN CAFDRS 112
SECTION 3.6 SUMMARY OF RESULTS: 113

CHAPTER 4: GENOTYPING RESULTS 114
SECTION 4.1: RS2072668 GENOTYPE (HOGG1) 115
4.1.1 Overall association between genotype and case/control status 116
4.1.2 Assessment of Gender on rs2072668 risk for pancreatic cancer 117
4.1.3 Assessment of Age on rs2072668 risk for pancreatic cancer: 118
4.1.4 Smoking status: 119
4.1.5 Smoking intensity 120
4.1.6 Family history of malignancy 121
4.1.7 Logistic regression 122
SECTION 4.2: XRCC1 (ARG399GLN) RS25487 123
4.2.1 Overall association between genotype and case-control status 123
4.2.2 Gender sub-group analysis: 125
4.2.3 Age sub-grouping 126

4.2.4 Smoking status ... *127*
4.2.5 Smoking Intensity ... *128*
4.2.6 Family history of malignancy ... *129*
4.2.7 Binary Logistic regression: ... *130*
SECTION 4.3 XRCCARG194TRP RS1799782 ... 131
4.3.1: Overall association between genotype and case-control status. ... *131*
4.3.2 Sub-group analysis: Gender ... *132*
4.3.3 Age sub-groupings: ... *133*
4.3.4 Analysis by smoking status ... *134*
4.3.5 Smoking intensity ... *135*
4.3.6 Analysis for family history sub groups: ... *136*
4.3.7 Logistic regression model: ... *137*
SECTION 4.4 XRCC 1 ARG280HIS ... 138
4.4.1 Overall association between genotype and case-control status ... *138*
4.4.2 Gender subgroup analysis: ... *139*
4.4.3 Age category subgrouping ... *140*
4.4.4 Smoking exposure sub groups ... *141*
4.4.5 Smoking intensity ... *142*
4.4.6 Family history sub groupings ... *143*
4.4.7 Logistic regression ... *143*
SECTION 4.5: APE1 ASP148GLN RS1130409 ... 145
4.5.1 Overall associations between genotype and case-control status ... *146*
4.5.2 Gender subgroup and risk ... *147*
4.5.3 Age sub-group and risk ... *148*
4.5.4 Smoking subgroups and risk ... *149*
4.5.5 Smoking intensity ... *150*
4.5.6 Family history sub groups and risk ... *151*
4.5.7 Logistic regression: ... *152*
SECTION 4.6 LOGISTIC REGRESSION INCLUDING ALL GENOTYPES ... 153
CHAPTER 5: MAIN FINDINGS AND DISCUSSION ... 155
SECTION 5.1: MAIN FINDINGS ... 156
SECTION 5.2 DISCUSSION: ... 157
5.2.1 Family history and tobacco exposure ... *158*
5.2.2 SNP analysis ... *162*
hOGG1 ... *162*
XRCC1 Arg399Gln ... *163*
XRCC1 Arg194Trp ... *165*
XRCC1 Arg280His ... *166*
APE1Asp148Gln ... *167*
SECTION 5.3: SUMMARY AND FURTHER INVESTIGATION ... 168
REFERENCES ... 173
PUBLICATIONS ... 201
APPENDICES ... 204
Appendix 1: Studies of hOGG1 polymorphisms and risk of malignancy ... *205*
Appendix 2: Studies of APE1/APEX1 polymorphisms and risk of malignancy ... *212*
Appendix 3: Selected studies of XRCC1 Arg194Trp polymorphisms and risk of malignancy ... *213*

Appendix 4: Tobacco smoking and pancreatic cancer..... 214
Appendix 5: MONICA tobacco smoking questionnaire..... 227

List of Tables and Figures

Chapter 1

Figure 1.1: Cancer Incidence, Mortality and Prevalence Worldwide IARC CancerBase 2004

Figure 1.2 Office of national statistics data – Incidence of pancreas cancer UK 2003

Figure 1.3 From: Parkin, D.M., Whelan, S.L., Ferlay, J., and Storm, H. Cancer Incidence in Five Continents, Vol. I to VIII IARC CancerBase No. 7, Lyon, 2005

Figure 1.4: Progression of precursor lesions to invasive cancer (Hruban et al., 2000)

Figure 1.5: Base Excision Repair

Figure 1.6: BER - Long and short patch (Dianov et al., 2003)

Figure 1.7: Nucleotide Excision Repair

Figure 1.8: Double strand break repair:

Figure 1.9: Mismatch repair

Table 1.1: AJCC/TNM staging of Pancreas adenocarcinoma

Table 1.2: Hereditary syndromes or diseases associated with pancreatic cancer

Table 1.3: Health consequences of tobacco smoking (From Harrison's Principles of internal medicine 15th Edition)

Table 1.4: Frequencies of genetic alterations in infiltrating ductal adenocarcinoma and its precursors

Table 1.5: Classification of neoplastic pancreatic cysts

Table 1.6: Inherited defects in DNA repair mechanisms and consequent illness

Figure 1.10: The role of inter-individual variation in human carcinogenesis

Chapter 2

Figure 2.1: hOGG1 Ser326Cys PCR-RFLP

Figure 2.2: XRCC Arg194Try PCR-RFLP

Figure 2.3: XRCC Arg399Gln PCR-RFLP

Figure 2.4: XRCC Arg280His PCR-RFLP

Table 2.1: Genotyping details - PCR and digestion conditions for individual genes

Chapter 3

Fig 3.1: Chart depicting enrolment and mode of diagnosis of cases

Fig 3.2: Chart depicting enrolment and inclusion of controls into study

Table 3.1: Gender grouping amongst cases/controls

Table 3.2: Tobacco smoking status

Table 3.3: Tobacco exposure (pack-years of smoking) Cases and Controls

Table 3.4: Family history of malignancy amongst Cases and Controls

Figure 3.3: Types of malignancies in caFDR+ (n=78) & coFDR+ (n=39)

Figure 3.4: Frequency of Tobacco smokers - Cases and Controls

Table 3.5: Detailed tobacco exposure data - cases and controls stratified by Family history

Table 3.6: Age of Onset of adenocarcinoma of pancreas [Mean (SD) years]

Chapter 4

Table 4.1: rs2072668 genotype frequency

Table 4.2: rs2072668 Homozygous wild-type vs. Carriers of variant allele

Table 4.3: Comparison of genotype frequencies for rs2072668 in male and female cases and controls

Table 4.4: rs2072668 genotypes in cases and controls; effect of age

Table 4.5: rs2072668 genotypes and susceptibility to pancreas cancer - effect of smoking status

Table 4.6: rs2072668 genotypes in cases and controls - effect of smoking intensity

Table 4.7: rs2072668 genotypes in cases and controls - effect of family history of malignancy

Table 4.8: Logistic regression analysis on hOGG1 genotype and relationship to pancreas cancer susceptibility

Table 4.9: XRCC1Arg399Gln genotype frequency

Table 4.10: XRCC1 Arg399Gln genotype: Variant allele carriers compared with homozygous wild-type types

Table 4.11: XRCC1 Arg399Gln Genotypes - effect of gender

Table 4.12: XRCC1 ARg399Gln genotype - effect of age

Table 4.13: XRCC1 Arg399Gln - Smoking status

Table 4.14: XRCC! ARg399Gln - effect of smoking intensity

Table 4.15: Xrcc1 Arg280His - effect of family history of malignancy

Table 4.16: Logistic regression for XRCC1 Arg399Gln for susceptibility to pancreatic cancer

Table 4.17: XRCC1 Arg194Trp genotype frequencies

Table 4.18: XRCC1 Arg194Trp genotypes - frequencies amongst cases and controls

Table 4.19: XRCC1 Arg194Trp - effect of gender

Table 4-20: XRCC1 Arg194Trp - effect of age

Table 4.21: XRCC1 ARg194Trp - effect of smoking status

Table 4.22: XRCC1 Arg194Gln - effect of tobacco smoking intensity

Table 4.23: XRCC1 Arg194Trp - effect of family history of malignancy

Table 4.24: Logistic regression on XRCC1 Arg194Trp genotype and relationship to pancreatic cancer susceptibility

Table 4.25: XRCC1 Arg280His genotype frequencies amongst cases and controls

Table 4.26: XRCC1 Arg280His - variant allele risk for pancreatic cancer

Table 4.27: XRCC1 Arg280His - effect of gender

Table 4.28: XRCC1 Arg280His - effect of age

Table 4.29: XRCC1 ARg280His - effect of smoking status

Table 4.30: XRCC1 Arg280His - effect of intensity of tobacco smoking

Table 4.31: XRCC1 Arg280His - effect of family history of malignancy

Table 4.32: Logistic regression on XRCC1 Arg280His and relationship to pancreatic cancer susceptibility

Table 4.33: APE1 Asp148Gln overall frequencies

Table 4.34: APE1 Asp148Gln genotype frequencies amongst cases and controls

Table 4.35: Ape1 Asp148Gln - gender sub-grouping

Table 4.36: APE1 Asp148Gln - age sub-grouping

Table 4.37: APE1 Asp148Gln - smoking status groups

Table 4.38: APE1 Asp148Gln - smoking intensity sub-groups

Table 4.39: APE1 Asp148Gln - family history of cancer sub-groups

Table 4.40: Logistic regression on APE1 Asp148Gln and relationship to pancreatic cancer susceptibility

Table 4.41: Logistic regression including all studied genotypes and risk for pancreatic cancer

Chapter 1: Introduction

Section 1.1: Pancreatic cancer - Epidemiology

Pancreatic cancer is the 13th most common malignancy worldwide, but is the 8th commonest cause of cancer related mortality worldwide (Fig 1.1) (Parkin et al., 2005). Most cases are diagnosed in the developed world (61%), where overall incidence and mortality rates are between 7 and 9 per 100,000 in men and 4.5 and 6 per 100,000 in women, with lower rates of disease identification in developing countries. It is the 5th most common cause of cancer related death in the developed countries. There are significant regional differences in the incidence worldwide with the highest incidence being reported in New Zealand Maoris (Phillips et al., 2002), native Hawaiians and black Americans and low rates from the Indian subcontinent and Nigeria (Boyle et al., 1989). Most (80-90%) cases are diagnosed when they are unresectable. Thus the survival is extremely low with a case fatality ratio approaching 0.99 (Rosewicz and Wiedenmann, 1997, Yeo and Cameron, 1999).

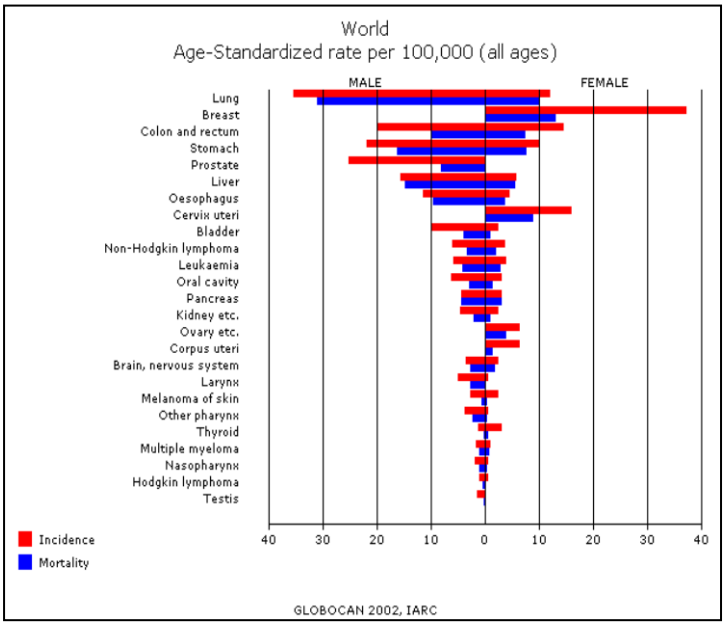

Figure 1.1: Cancer Incidence, Mortality and Prevalence Worldwide IARC CancerBase 2004

In the UK and Ireland, pancreatic cancer was the 9th and 10th commonest cancer respectively in men and women in the 1990's - age-standardised incidence rates were 10.5 per 100,000 in males and 7.8 per 100,000 in females. In 2003 there were 2878 males and 3021 females diagnosed with the malignancy - age standardized incidence rates of 11.8 and 11.9 respectively [ONS Cancer: number of new cases 2003, sex and age at *http://www.statistics.gov.uk/statbase/ssdataset.aspvlnk=9096*].

Pancreatic cancer is rare under 45 yrs. The incidence increases with age and males predominate in all age groups up to 70 years. However the incidence increases in women beyond this age group (Fig: 1.2).

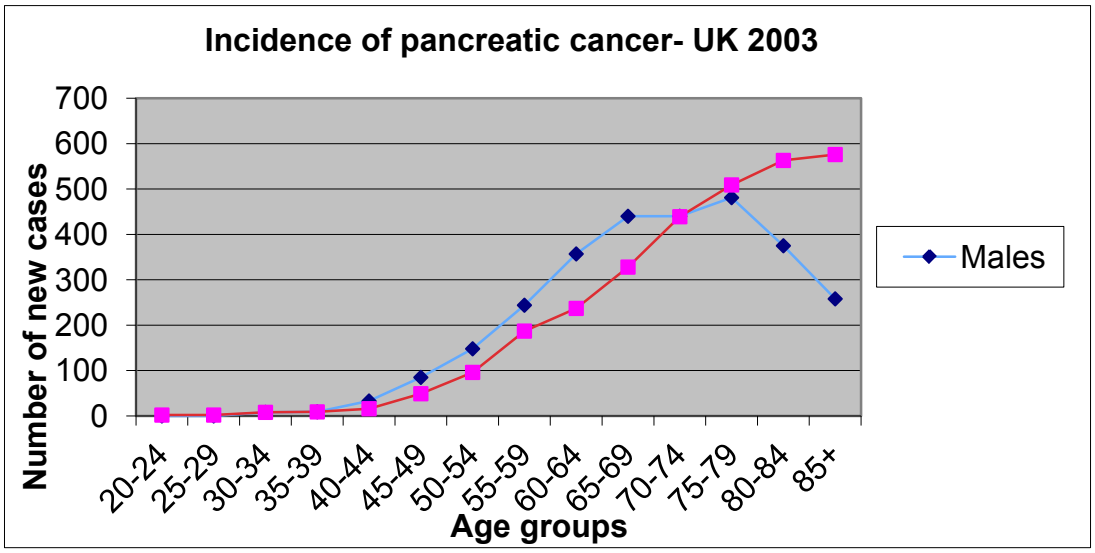

Figure 1.2 Office of national statistics data – Incidence of pancreas cancer UK 2003

Based on data from 1994-97 the lifetime risk (the risk to an individual that pancreatic cancer will occur at any time without regard to the time/age at which it will occur) of being diagnosed with pancreatic cancer was 1 for males and 1.1 for females in England and Wales (Quinn MJ et al., 2000). Reflecting the worldwide picture, survival rates are lower than for most other cancers. This is due to the advanced presentation and limited treatment opportunities available for effective treatment. For cases diagnosed during

1996-99, the one year relative survival was 13% and 5 year survival was 2-3% [ONS. Cancer Survival: England and Wales, 1991-2000 {*http://www.statistics.gov.uk/statbase/ssdataset.asp?vlnk=7899]*.

Section 1.2: Pancreatic cancer - Clinical features, Management and Outcome

1.2.1 Clinical Features

Pancreatic ductal adenocarcinoma is a disease with a poor prognosis. The most important reason for this is its late presentation. The factors responsible for this are

- Its retro-peritoneal situation
- Lack of a specific symptom in the early stages of tumour growth
- The absence of a specific tumour marker

The presenting symptoms of pancreatic cancer depend on the location of the tumour within the gland, as well as on the stage of the disease. The organ extends from the "C" of the duodenum to the hilum of the spleen. The majority of tumours develop in the head of the pancreas on the right side of the portal vein and cause obstructive jaundice, which is typically painless. Vague abdominal discomfort and nausea are also common. Some patients may describe intermittent pain situated in the epigastrium, occasionally predating the onset of jaundice. However this is fleeting and most patients do not present with this as their main symptom. Late in the course of the disease they can encroach and invade the portal vein and mesenteric artery. Cancers to the left of the portal vein are asymptomatic until they cause pain (Nakakura and Yeo, 2006). This is due to infiltration of the retroperitoneal neural plexuses by the tumour before it encroaches on to the bile duct as compared to those on the right of the vein. The latter tumours being closer to the bile duct result in its early involvement and obstructive jaundice. Locally advanced pancreatic cancer causes dull, deep upper abdominal and back pain by invasion of the retroperitoneal neural structures.

More rarely, a pancreatic tumour may also cause duodenal obstruction or gastrointestinal bleeding. A striking feature of pancreas cancer is the significant weight loss associated with it (Wigmore et al., 1997a). Obstruction to the pancreatic duct causing exocrine insufficiency plays a definite role in this. There are also yet unidentified tumour related factors, which suppress appetite and cause weight loss (Cariuk et al., 1997, Wigmore et al., 1997b). Diabetes is associated with this malignancy both as an early manifestation and as an etiologic factor (Ben et al., 2011), pro-thrombotic tendency is a feature and may present with venous thrombosis (Epstein and O'Reilly, 2012). Acanthosis nigricans is another para-neoplastic feature, which is seen as black pigmentation in the flexures of the axillae (Thrash et al., 2013, Shah et al., 2013).

By far the commonest tumour arising in the pancreas is a ductal adenocarcinoma; the other carcinomas are acinar cell carcinoma and adenosquamous differentiation. Other histological types are lymphoma and metastases especially from breast and renal cancers.

1.2.2 Assessment, Diagnosis and Staging:

Physical examination apart from jaundice may be quite unremarkable. Other clinical findings may include evidence of recent weight loss, cervical lymphadenopathy (Troiser's sign), hepatomegaly, and ascites. The latter signs correlate significantly with advanced disease. Results of routine blood tests are generally nonspecific and may include mild abnormalities in liver-function tests, hyperglycaemia, and anaemia. Evaluation of a patient in whom pancreatic cancer is suspected should focus on diagnosis and staging of the disease, assessment of resectability, and palliation of symptoms. Multiphase (arterial, portal and venous), multi-detector (spiral/helical)

computed tomography (CT) with oral and intravenous administration of contrast material with thin sections (3mm) is the imaging procedure of choice for the initial evaluation(McNulty et al., 2001). This technique allows visualization of the primary tumour in relation to the superior mesenteric artery, celiac axis, superior mesenteric vein, and portal vein and also its relationship to adjacent organs (Mansfield et al., 2008). It also assesses the liver comprehensively. Overall, contrast-enhanced CT predicts surgical resectability with 80 to 90% accuracy (Karmazanovsky et al., 2005).

Endoscopic ultrasound (EUS) is useful in patients in whom pancreatic cancer is suspected although there is no visible mass identifiable on CT (Rafique et al., 2007). It is the preferred method of obtaining tissue for diagnostic purposes. Although a tissue diagnosis is not needed in patients who are scheduled for surgery, it is required before the initiation of chemotherapy or radiation therapy. Endoscopic retrograde cholangio-pancreatography (ERCP) shows the pancreatic and biliary ductal anatomy and can be used for purposes of biliary stent insertion and brushing, which provides tissue for diagnosis. We do not recommend this technique for purposes of diagnosis of pancreatic cancer in the first instance. Magnetic Resonance (MR) imaging is being increasingly used in the characterization of pancreatic masses (Shrikhande et al., 2012), especially in the follow-up of incidental lesions. This is not a usual mode of investigation in the work-up of pancreas cancer.

In patients who have large tumours, especially in the body and tail of the pancreas, as well as other indications of advanced disease such as weight loss, an elevated level of carbohydrate antigen 19-9 (CA 19-9), ascites, or equivocal CT findings, a staging laparoscopy with laparoscopic ultrasound can accurately determine vascular involvement, peritoneal and metastatic disease.

Pancreatic cancer is staged according to the most recent edition of the American Joint Committee on Cancer tumour–node–metastasis classification, which is based on assessment of resectability by means of helical CT (Table 1.1) (Sobin et al., 2009). T1, T2, and T3 tumours are potentially resectable, whereas T4 tumours, which involve the superior mesenteric artery or celiac axis, are unresectable.

Table 1.1: AJCC/TNM staging of Pancreas adenocarcinoma (Sobin et al., 2009)

Stage	Tumour stage	Node stage	Distant metastasis	Characteristics	Median Survival (months)
1A	T1	N0	M0	Lesion in pancreas, ≤ 2 cms	24.1
1B	T2	N0	M0	Tumour in pancreas, ≥ 2 cms	20.6
2A	T3	N0	M0	Tumour beyond pancreas but does not involve SMA or CA	15.4
2B	T1, T2 or T3	N1	M0	Regional Ln metastases	12.7
3	T4	N0 or N1	M0	Tumour involving SMA and or CA – unresectable disease	10.6
4	T1, T2, T3 or T4	N0 or N1	M1	Distant metastasis	4.5

1.2.3 Management and outcome

Patients with pancreatic cancer are best cared for by multidisciplinary teams that include surgeons, medical and radiation oncologists, radiologists, gastroenterologists, nutritionists, and pain specialists, among others (Pawlik et al., 2008).

1.2.3.1 Biliary decompression:

Up to 70% of patients with pancreatic cancer present with biliary obstruction, which can be relieved by percutaneous or endoscopic stent placement. However in patients with a resectable malignant CBD stricture, insertion of a plastic biliary stent followed by delayed surgery is associated with a higher morbidity compared with early surgery and two RCTs have shown that overall morbidity was increased if plastic biliary drains were placed preoperatively compared with direct surgery (Lai et al., 1994, van der Gaag et al., 2010), this was however not confirmed by a Cochrane analysis (Wang et al., 2008) Preoperative drainage of potentially resectable malignant CBD obstruction in indicated only in patients who are candidates for neoadjuvant therapies, in patients with acute cholangitis, or in patients with intense pruritus and in whom delayed surgery is indicated (Dumonceau et al., 2012). Biliary stenting at Endoscopic retrograde cholangio-pancreatography is the preferred route (Moss et al., 2007b) for distal biliary strictures, which is the usual site of obstruction with head of pancreas cancers. ERCP is however not possible in rare occasions when the duodenum is distorted and or narrowed and the ampulla is not accessible endoscopically. Percutaneous approach is adopted in these situations; however this route is associated with an increased risk of complications. Plastic biliary stents have generally been preferred, however there is a recent trend towards short wide metal stent insertion (Moss et al., 2007a, Dumonceau et al., 2012).

Decompression is appropriate for patients in whom surgery is delayed (Tol et al., 2012), such as patients who are treated with neo-adjuvant therapy before resection or who are referred to other centres for treatment. Patients with symptoms of cholangitis require decompression as well as antibiotic treatment before surgery. A recent multi-centre randomized clinical trial from Netherlands has concluded that the delay associated with pre-operative biliary drainage [Mean times from randomization to surgery were 1.2 (0.9-1.5) and 5.1 (4.8-5.5) weeks in the early surgery group and pre-operatively biliary drained group groups, respectively ($p < 0.001$)] does not impact survival (Eshuis et al., 2010) but increases the risk of serious complications (Eshuis et al., 2010). An earlier study from our Unit has identified that bilirubin increases by an average of approximately 100 μmol/l/week (Mansfield et al., 2006) and is supportive of the argument for prompt action and fast-track surgery in suitable patients (French et al., 2009).

1.2.3.2 Resectable pancreas cancer:

For patients with resectable disease i.e. broadly stage 1 and 2A, surgery remains the treatment of choice (Lillemoe, 1995). Depending on the location of the tumour, the operative procedures may involve pancreatico-duodenectomy (the Whipple operation), distal pancreatectomy, or total pancreatectomy. A minimum of 12 to 15 lymph nodes should be resected (Slidell et al., 2008), and every attempt should be made to obtain a tumour-free margin. Data from several randomized clinical trials indicate that a more extensive resection does not improve survival but increases postoperative morbidity (Yeo et al., 2002) (Michalski et al., 2007). Recent studies show that the results of vein resection and vascular reconstruction in patients with limited involvement of the superior mesenteric vein and portal vein are similar to the results in patients without vein involvement (Tseng et al., 2006) (Chua and Saxena, 2010) and indeed a recent

report (n=34) suggests that portal vein resection should be a routine part of pancreatico-duodenectomy for cancer (Turrini et al., 2013).

There are several factors which have positive influences on survival after pancreas resection: well-differentiated tumour, lower tumour stage, no duodenal or major vascular invasion, no perineural invasion, negative lymph node metastases (Lim et al., 2003), R0 resection (Lewis et al., 2013), surgery at a high-volume centre (Schmidt et al., 2010) (Garcea et al., 2008a) (Berger et al., 2008) and low peri-operative transfusion requirements (Kneuertz et al., 2011).

The following factors have no influence on disease-specific survival after pancreas resection: age (Riall, 2009) and extending the resection beyond that described above (Orr, 2010).

Post-operative adjuvant treatment is beneficial and adjuvant chemotherapy after R0 resection is standard. The unequivocal demonstration that postoperative treatment improves the outcome in these patients is one of the most important advances that has been made in the management of pancreatic cancer. A review has reported a 30% resection rate with a 5% hospital mortality (Wolff, 2003) in specialized pancreatic centres with a 5-year survival rate between 1-20%. Median survival of patients who undergo resection was 13.5 months (Yeo et al., 1997), the same randomized trial showed a statistically significant increase in survival to 19.5 months when post-operative chemo-radiation was used. A review of randomized trials has however failed to confirm the survival advantage of this modality of treatment over surgery alone (Stojadinovic et al., 2003). The ESPAC-1 trial has shown that the use of adjuvant 5-FU and Folinic acid was associated with a survival advantage (Neoptolemos et al., 2003a). The median survival was 23.2 (95% CI 20.1 – 26.5) months in the ESPAC-1 adjuvant

5-FU and Folinic acid arm Vs. 16.8 (95% CI 14.3 – 19.2) months in surgery alone arm (Neoptolemos et al., 2003b). Similar results have come out of the Charite Onkologie 1 trial. The ESPAC-3 trial (Neoptolemos et al., 2003b, Oettle, 2003) compared 5FU + Folinic acid Vs. Gemcitabine as adjuvant treatment following pancreatic resection for adenocarcinoma and no difference in disease free survival, overall survival [(5FU: 23 (95% CI 21.1 -25 months), Gemcitabine: 23.6 (95% CI 21.4 – 26.4)] or global quality of life scores between the treatment groups. However, 77 (14%) receiving fluorouracil plus Folinic acid had 97 treatment-related serious adverse events, compared with 40 patients (7.5%) receiving gemcitabine, who had 52 events ($P < .001$). Nevertheless, only a few patients survive for at least 5 years after R0-resection and adjuvant chemotherapy. The ESPAC-4 trial (scheduled to close on 11/01/2014) compares gemcitabine alone against combination therapy of gemcitabine plus capecitabine in patients within one year of a potentially curative resection.

A recent approach in patients with resectable pancreatic cancer is the use of preoperative (neoadjuvant) treatment. Nonrandomized, phase 2 studies suggest that this approach is at least as effective as postoperative treatment and may decrease the rate of local failures and positive resection margins after surgery (Evans et al., 2008, Varadhachary et al., 2008). These findings are particularly relevant for patients who have so called “borderline resectable” tumours with limited vascular involvement; in these patients, preoperative treatment may result in tumour-free resection margins (Katz et al., 2008). The evidence for neo-adjuvant strategies for pancreatic cancer is further supported by a recent computational modelling of pancreas cancer kinetics (Haeno et al., 2012). In a unique study involving 2 groups of patients – one from rapid autopsy participants which led to a mathematical model to understand growth dynamics of pancreas cancer cells, which was further validated in a cohort of patients who

underwent curative surgery followed by adjuvant therapy; suggested that aggressive systemic therapy should be offered early after diagnosis regardless of the stage of the disease given that systemic treatments which reduce tumour growth rate early in the course of the disease are superior to upfront tumour resection (Haeno et al., 2012).

In patients who do not undergo resection the survival is poor and this scenario is common. A recent review (Mossner, 2010) titled "What's new in therapy of pancreas cancer?" began by stating, "The title of this review is more promising in comparison to reality…" Majority of these cancers present at advanced stages when palliative therapy is the only option. About 80% of patients are un-resectable at presentation (Fischer et al., 2003). Overall 5-year survival for this cancer is around 4% (Andre et al., 1998). Median survival after diagnosis for the vast majority of patients is around 6 months and most patients need palliative treatment.

1.2.3.3 Loco-regional disease:

This is disease, which involves the portomesenteric vasculature (extensive venous encasement and/ arterial involvement) with or without significant peri-pancreatic lymphadenopathy. Approximately 30% of patients with pancreatic cancer present with advanced loco- regional disease, and an additional 30% of patients will have local recurrence of tumours after treatment for early disease. Management options range from systemic chemotherapy alone to combined forms of treatment with chemo-radiation therapy and chemotherapy. A series of randomized trials conducted over the past two decades suggested that that chemotherapy was superior to best supportive care in these patients (Huguet et al., 2007, Sultana et al., 2007). Chemotherapy is indeed the critical component in the treatment approach (with gemcitabine-based approaches deemed more effective) and combined treatment with chemotherapy and radiation therapy is an

effective, though more toxic, approach (Ciliberto et al., 2013). However, randomized clinical trials of such combined treatments have had low recruitment, precluding a firm conclusion. Recently FOLFIRINOX – a combination regime of oxaliplatin, irinotecan, fluorouracil, and leucovorin has been found more effective than gemcitabine alone (Conroy et al., 2011) in locally advanced (Peddi et al., 2012) and metastatic scenarios.

1.2.3.4 Metastatic disease:

The majority of patients experience metastatic disease, mainly in the liver and peritoneal cavity. The treatment of these patients with advanced disease remains palliative, and these patients should be offered the opportunity to participate in clinical trials evaluating new treatments when available. A meta-analysis of published findings from clinical trials showed an improvement in survival among patients who received chemotherapy; these findings suggest that active treatment is beneficial (Sultana et al., 2007). Gemcitabine has been the treatment of choice on the basis of the results of the randomized trial of gemcitabine versus fluorouracil (Burris et al., 1997). Multiple new agents with diverse mechanisms of action in combination with gemcitabine have been tested in randomized clinical trials, with no improvement in outcome. Capecitabine has been used in such a combination (GemCap) demonstrated a significant survival benefit (Cunningham et al., 2009b) The only agent that, in combination with gemcitabine, has shown a small, but statistically significant improvement in survival among patients with advanced pancreatic cancer is erlotinib, an inhibitor of the epidermal growth factor receptor (EGFR) (Moore et al., 2007). However, the high frequency of KRAS2 mutations in pancreatic cancer probably limits the benefits of an EGFR inhibitor (Hidalgo, 2010). As compared with erlotinib alone, the combination of gemcitabine and

erlotinib has more toxicity, particularly gastrointestinal symptoms. Together with the rather modest improvement in survival (about 14 days), the toxicity of this combination has limited its wide acceptance as the standard of care. A recent meta-analysis of randomized trials showed that patients with minimal disease-related symptoms and otherwise good health may benefit from combination chemotherapy with gemcitabine and either a platinum agent or a fluoropyrimidine (Heinemann et al., 2008, Cunningham et al., 2009a). Therefore the accepted treatment approach for patients with advanced disease is either gemcitabine given alone or gemcitabine combined with a platinum agent (oxaliplatin) (Louvet et al., 2005), erlotinib, or a fluoropyrimidine (5FU, capecitabine) (Hidalgo, 2010).

Best supportive care includes nutritional support, therapy for pain, treatment of jaundice, duodenal obstruction and pain. A systematic review concluded that, in patients who are found to have unresectable disease at laparotomy prophylactic gastroenterostomy is indicated (Huser et al., 2009). Palliation of biliary obstruction with plastic stents is acceptable in patients with disseminated disease who have a short life expectancy. Stents for relief of duodenal obstruction can also be used in a similar patient for short-term relief of obstruction. However, most evidence favours surgical bypass of biliary and duodenal obstruction when life expectancy is considered to be more than a few months (Olgyai and Olah, 2007) (Jeurnink et al., 2007).

Thus it is quite apparent that the best approach towards this malignancy is prevention. Its relationship with smoking [25% (Fuchs et al., 1996), 26% (in Whites) and 29% (in Blacks) (Silverman et al., 1994) of all pancreatic cancers are attributable to tobacco smoking] , which is potentially the most important avoidable cancer risk in humans, provides us with a unique opportunity to reduce incidence. This relationship also provides an opportunity to examine the role of carcinogens and their role in the

development of a particular cancer. Better understanding of the mechanism of pancreatic carcinogenesis will contribute in the early diagnosis and improve outcome.

Section 1.3: Major risk factors in the development of pancreatic cancer

The causes of pancreatic cancer remain unknown. The major risk factors for pancreatic cancer are increasing age, tobacco smoking (HAMMOND, 1964, 2004) and family history of the cancer (Li et al., 2004). Several environmental factors have been implicated, but evidence of a causative role exists only for tobacco use. Recently an increased risk has been observed among patients with blood type A, B, or AB as compared with blood type O (Iodice et al., 2010, Wolpin et al., 2010).

1.3.1 Heritable causes and Familial pancreatic cancer

One of the greatest risk factors for pancreatic adenocarcinoma is a family history of the disease. A family with pancreas adenocarcinoma was described as early as in the 1970's. In 1987 Ehrenthral described a family with 3 successive generations of women with pancreas cancer. About 5-10 % of cases demonstrate a familial tendency and in 10-20% of cases a heritable factor may play a significant role in causation (Petersen and Hruban, 2003). Also it is now well established that a family history of any type of cancer increases the risk for pancreas cancer (Ghadirian et al., 1991, Silverman et al., 1999).

Broadly it is felt that there are 2 categories of hereditary risk for pancreatic cancer

1) Germline mutations and Pancreatic cancer (Table 1.2): Of pancreatic cancer cases, 5-10% are part of a well-defined cancer-predisposing syndrome for which germ-line genetic alterations are known (Habbe et al., 2006).

2) Familial Pancreatic cancer: Although there is no agreed definition, Familial pancreatic cancer is accepted as an inherited predisposition based on family clustering

in families in which there are multiple first and second degree relatives with ductal pancreatic adenocarcinoma in the absence of a known genetic susceptibility syndrome (Brentnall, 2005). FPC occurs at an earlier age (Petersen et al., 2006) is clustered in families (Pogue-Geile et al., 2006, Earl et al., 2006) and has the same poor prognosis as its sporadic counterpart.

Table 1.2: Hereditary syndromes or diseases associated with pancreatic cancer

Syndrome	Genetic defect	Reference
Hereditary pancreatitis	Mutation in the cationic trypsinogen gene (PRSS1)	(Howes et al., 2004, Whitcomb et al., 1999)
Peutz-Jeghers Syndrome	Germ line mutation in the tumour suppressor gene STK11/LKB1	(Yee et al., 2003, Su et al., 1999)
Familial atypical multiple mole melanoma	Germ line mutation of the p16 tumour suppressor gene	(Lynch and Fusaro, 1991, Rulyak et al., 2003a)
Cystic fibrosis	Mutation in the cystic fibrosis trans membrane regulator (CFTR) gene	(McWilliams et al., 2005a, Sheldon et al., 1993)
Familial ovarian and breast cancer	Germ line mutations of BRCA2 & BRCA1	(van Asperen et al., 2005, Lubinski et al., 2004, Risinger et al., 1996)
Hereditary non-polyposis colorectal cancer	Germ line mutations in DNA mismatch repair genes - MLH1, MSH2, MSH6, PMS1, PMS2	(Yamamoto et al., 2001, Ghimenti et al., 1999)
Ataxia Telangiectasia	Ataxia telangiectasia mutated (ATM) gene	(Yu et al., 2004a, Li et al., 2006a)
Li-Fraumeni	Germ line mutation of tumour suppressor p53	(Flanders and Foulkes, 1996, Lefrou et al., 2006)
Familial adenomatous polyposis	Germ line mutations in the adenomatous polyposis coli (APC) gene	(Gupta and Mazzara, 2005, Fendrich and Bartsch, 2005)
Familial Pancreatic Cancer	Unidentified	(Habbe et al., 2006, Brentnall, 2005)

1.3.1.1 Hereditary Pancreatitis:

This is an illness, which typically begins, in adolescent children who present with recurrent episodes of pancreatitis. This can result in chronic pancreatitis leading to subsequent pancreatic insufficiency (endocrine and exocrine) and chronic pain. The age of onset is a clear distinguishable feature of this condition.

Gain of function in the cationic trypsinogen gene (PRSS1) due to mutations – over 25 have been identified and R122H and N291 are the commonest (Finch et al., 1997), results in premature activation or ineffective denaturation of trypsin leading to parenchymal injury. Nearly half of these patients go on to develop chronic pancreatitis and there is a 40% risk of developing pancreas cancer in these individuals. Compared to the general population they have a 54-times higher risk, and in those who smoke tobacco this risk increases to 154-times (Lowenfels et al., 1997).

1.3.1.2 Cystic Fibrosis:

This autosomal recessive disease occurs due to a mutation in the cystic fibrosis trans membrane conductance regulator (CFTR) gene (located on 7q31.2), which codes for a chloride transport channel across the cell membrane. More than 1000 mutations in this gene have been described in people with Cystic Fibrosis. Prevalence of the gene is 2-4% - it is the most common inherited lethal disease amongst Caucasians and in Europe 1 in 2000-3000 new borns is found to be affected by CF (WHO, 2010). Due to improvements in management of the respiratory manifestations of this disease, patients are now surviving longer and reaching their late thirties (Jackson et al., 2011) (http://www.cff.org/aboutCFFoundation/NewsEvents/2006NewsArchive/2711.cfm, 2006).

Risk for pancreas cancer in this cohort of patients has been difficult to determine given the various causes of mortality in them. Risk estimates have varied from 2.6 fold increased risk (CF foundation data) (Maisonneuve et al., 2003) through 5.3 (SEER data) (Maisonneuve et al., 2007) and up to 31.5 times (Neglia et al., 1995) increased risk for development of pancreatic cancer. Carrying a CFTR mutation resulted in a very modest increased risk of 1.4 (1.04 – 1.89) for pancreas cancer (McWilliams et al., 2010).

1.3.1.3 Peutz-Jeghers Syndrome (PJS)

PJS is an autosomal dominant condition characterised by hamartomatous gastrointestinal polyps and muco-cutaneous, pigmented lesions caused by a mutation in the serine/threonine protein kinase 11 STK11/LKB1 gene. There is a variable phenotype and high penetrance. The prevalence is about 1 in 50,000. The hamartomatous polyps are most common in the small intestine (in order of prevalence: in the jejunum, ileum, and duodenum) but can also occur in the stomach, large bowel, and upper airways. Muco-cutaneous hyper-pigmentation presents in childhood as dark blue to dark brown macules around the mouth, eyes, and nostrils, in the peri-anal area, and on the buccal mucosa. Hyper-pigmented macules on the fingers are common. The macules may fade in puberty and adulthood.

Individuals with Peutz-Jeghers syndrome are at increased risk for a wide variety of epithelial malignancies (lung, colorectal, gastric, pancreatic, breast, ovarian, endometrial and testicular cancers). An important aspect of the management of PJS patients is screening for these malignancies. Although various screening protocols have been put forward for the above malignancies, the pancreas cancer programs have not been evaluated in trials.

A meta-analysis of PJS studies concluded a RR for pancreatic cancer of 132 and a lifetime risk of 36% (Giardiello et al., 2000) whilst a more recent study concluded a

much lower risk of 7% cumulative risk at 60 years of age. A multi-centre study including n=419 individuals with PJS revealed that eleven patients (6 males and 5 females) were diagnosed with pancreatic cancer and the risk of developing pancreatic cancer was 3%, 5%, 7%, and 11% at ages 40, 50, 60, and 70 years, respectively (Hearle et al., 2006). A systematic review (Beggs et al., 2010) has not found any evidence in favour for routine surveillance of these patients for pancreas cancer, furthermore routine surveillance and screening was expensive [USD 350000/ life saved (Latchford et al., 2006)].

1.3.1.4 Familial atypical multiple mole melanoma (FAMMM):

This is an autosomal dominant disorder, which is associated with various germ line mutations and variable penetrance. In addition to atypical moles, melanoma and pancreas cancer they are also at high risk for cancers of breast, endometrium and lung. Individuals who develop pancreatic cancer carry a CDKN2A mutation and this gene codes for p16, a cyclin-dependent kinase inhibitor (Lynch and Fusaro, 1991, Lynch et al., 2002). The lifetime risk by age 75 is 17% with mean age of onset of pancreas cancer at 58 years of age (Vasen et al., 2000).

A recent Endoscopic ultrasound based surveillance programme in asymptomatic mutation carriers amongst a Dutch family detected pancreatic tumours in all – 2 were invasive malignancies and the third was a side-branch IPMN (Kluijt et al., 2009). There is on-going debate on whether to screen and the best modality to use in these families.

1.3.1.5 Hereditary breast and ovarian cancer (HBOC)

Germ-line mutations in the *BRCA1* and *BRCA2* (*BRCA1/2*) tumour suppressor genes are highly penetrant for increased risks of breast and ovarian cancers. They play a key role in DNA damage repair and the maintenance of genomic stability and a recent

population-based study from Canada suggested that *BRCA1/2* mutations were associated with a significantly increased risk of cancers overall and at sites other than breast and ovary (Risch et al., 2006). The Standardized incidence rate for pancreas cancer in BRCA1 carriers was 2.55 (95% CI=1.03-5.31, P=0.04) and for BRCA2 carriers was 2.13 (95% CI=0.36-7.03, P=0.3) (Iqbal et al., 2012).

Other cancers that have been shown to be associated with *BRCA1/2* mutations include male breast cancer, prostate cancer, and melanoma. However these mutations also occur at increased frequency in families without a history of breast and/ovarian cancers but with pancreas cancer/familial pancreas cancer (Murphy et al., 2002, Couch et al., 2007).

1.3.1.6 Lynch Syndrome (Hereditary non-polyposis colorectal cancer HNPCC)

This is the most common form of hereditary colon cancer (Colas et al., 2012) secondary to mutations in mis-match repair genes – MSh2, MLH1 and less commonly MSh6, PMS1 and PMS2 (Martin-Lopez and Fishel, 2013). There are 2 forms described – Lynch 1 where colon cancers only occur and Lynch 2 which is associated with extra-colonic malignancies including stomach, small bowel, hepato-pancreato-biliary, breast, renal pelvis and ureter. The exact incidence and risk for pancreas cancer in this syndrome is unknown.

1.3.1.7 Familial adenomatous polyposis (FAP)

FAP results from a mutation in the APC gene, which is involved in cell signalling. FAP is not uncommon with an incidence reported to vary from 1:6,850 to 1:23,700 live births and leads to development of colorectal carcinoma in almost 100% of cases by 40 years of age. FAP is characterized by the formation of hundreds to thousands of colorectal adenomatous polyps. Although the development of colorectal cancer is the prevalent complication, FAP is a multisystem disorder of growth and individuals can

develop thyroid and pancreatic cancer, hepatoblastomas, CNS tumours (especially medulloblastomas), and various benign tumours such as adrenal adenomas, osteomas, desmoid tumours and dental abnormalities. Pancreatic tumours in FAP patients are rare. In a cohort study of 1,391 patients with FAP reported in the Johns Hopkins Registry, 4 patients were found to have developed a pancreatic adenocarcinoma (Giardiello et al., 1993). In view of the low prevalence of pancreatic cancer and the scarcity of published data, surveillance is not routinely recommended. Surveillance is however recommended for the prevention of other intestinal malignancies, such as duodenal ampullary carcinomas and gastric carcinomas.

1.3.1.8 Familial pancreatic cancer (FPC):

FPC is the term applied to families with two or more first-degree relatives who have been diagnosed with PC that is not associated with a known cancer syndrome. From a study at Johns Hopkins (Klein et al., 2004) it was found that any member of a kindred with a first-degree relative diagnosed with PC had a nine-fold increased risk of developing PC [OR 95% CI (4.5 – 16.1)]. Risk estimates varied by the number of PC-affected first-degree relatives: having one affected first-degree relative increased the risk to 4.6 (95% CI 0.5-16.4), two affected first-degree relatives gave a risk of 6.4 (95% CI 1.8-16.4) and three affected first-degree relatives increased the risk 32-fold (95% CI 10.2-74.7) (Klein et al., 2004).

Studies on members of "Family X" – 18 cases of pancreas cancer were identified in 4 generations – suggested that the susceptibility gene for FPC was the palladin gene at chromosome 4q32-34 (Eberle et al., 2002), however subsequent studies on other families has not confirmed this (Slater et al., 2007) . An interesting feature in "Family X" was the development of the cancer earlier in life in successive generations – "genetic

anticipation". In a recent European study (McFaul et al., 2006) the median age at death in successive generations from pancreas cancer was – 70, 64 and 49 years. Also cigarette smoking is associated with earlier development of the disease by 10 years as compared to non-smokers (Rulyak et al., 2003c).

Numerous modes of screening and its utility in the individuals belonging to FPC families have been studied and the role of EUS in demonstrating very small intra-pancreatic lesions has been confirmed (Kimmey et al., 2002, Canto, 2007, Canto et al., 2006). However on a background of the mortality and morbidity imposed by a pancreatic resection and the nearly 50% incidence of benign lesions (in individuals who underwent resection in these 2 studies) it is still not clear whether screening is beneficial and if it provides survival benefit. A systematic review and mathematical modelling of FPC kindreds comparing four separate management strategies for preventing PDAC in high risk individuals, namely, (1) prophylactic total pancreatectomy, (2) annual surveillance by EUS, (3) annual surveillance by EUS-FNA, (4) doing nothing concluded that the effectiveness of any screening program for FPC kindreds would depend greatly on the subsequent management of the 50% or more of individuals demonstrating findings of chronic pancreatitis by EUS (Rubenstein et al., 2007). Based on a 20% lifetime risk of PC, the investigators determined that the 'doing nothing' strategy actually provided the greatest quantity of remaining years of life, the greatest remaining quality-adjusted life years, and at the the lowest cost.

1.3.2 Age:

The most reliable predictor of sporadic pancreatic cancer is age. The risk of development correlates with increasing age (Aoki and Ogawa, 1978). The malignancy is extremely rare below 45 years (Morgan and Wormsley, 1977) and 80% cases occur between the ages of 60 and 80 (Ahlgren, 1996, Gold and Goldin, 1998). The risk for those in the 8th decade of life has been quoted to be 40 times that of those in the 4th

decade (Li, 2002). This increase in incidence as age advances has been documented in nearly all countries (Fig 1-3).

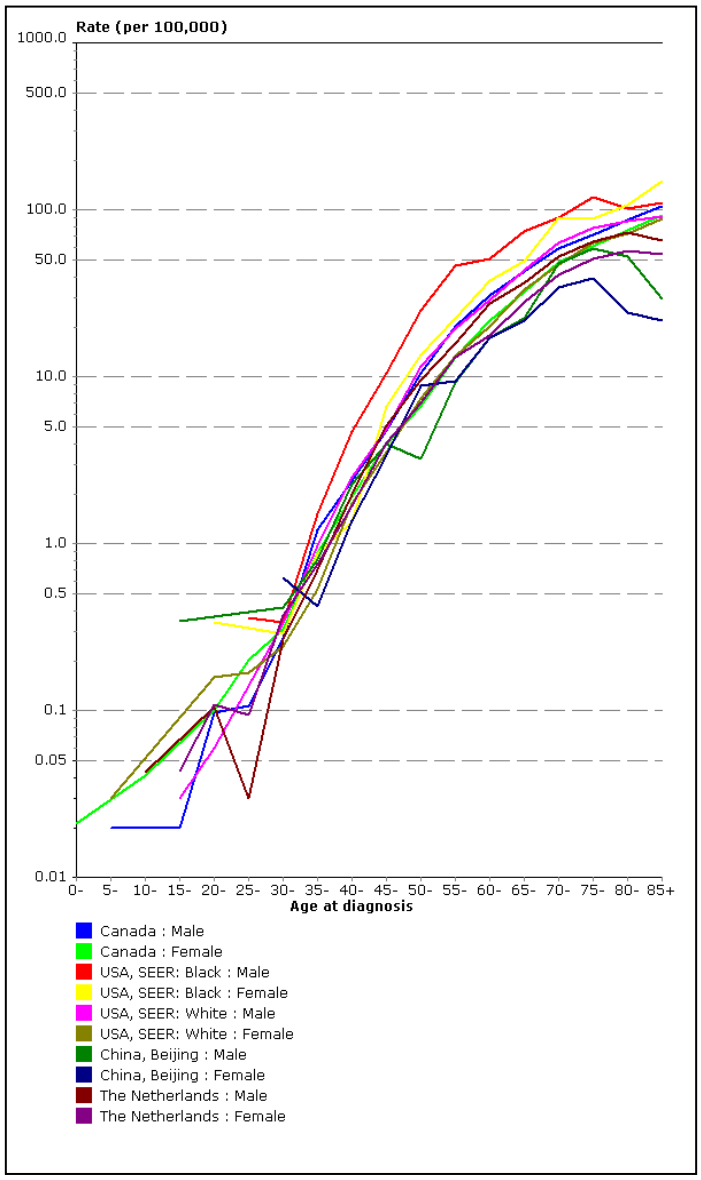

Figure 1.3 From: Parkin, D.M., Whelan, S.L., Ferlay, J., and Storm, H. Cancer Incidence in Five Continents, Vol. I to VIII IARC CancerBase No. 7, Lyon, 2005.

1.3.3 Race:

The Maoris (Fraumeni, 1975, Phillips et al., 2002) in New Zealand have the highest rates of pancreas cancer in the world and they do not show the increased incidence amongst men. Maori women have the highest rates of pancreas cancer amongst females in the world (Phillips et al., 2002). The high prevalence of smoking amongst Maoris

has been attributed to the high incidence of lung, pancreas and kidney cancers. In sharp contrast the numbers of bladder cancer, which is also a smoking related cancer is low amongst them (McCredie et al., 2000). High rates are also detected amongst black Americans (Garfinkel, 1991, McCarty, 2001) in the USA, which is also true for lung cancer (Stellman et al., 2003). The exact reason for this has not been ascertained. A large case-control study concluded that the increased levels of tobacco smoking did not explain the increased risk amongst black Americans (Silverman et al., 1994). A western diet rich in animal fat (McCarty, 2001), nutritional imbalances, high-risk occupations, limited access to medical care and other socio-economic factors associated with poverty (Greenberg and Schneider, 1995) in addition to cigarette smoking are thought to be responsible for the higher occurrence of pancreatic cancer in Americans blacks as compared to whites. Another contributing factor for these racial differences could be differences in the ability to metabolise tobacco derived carcinogens – e.g. differences between Caucasians and non-Caucasians with respect to urinary metabolites of tobacco derived carcinogens (Richie et al., 1997).

1.3.4 Gender:

There is a higher incidence amongst males as compared to females in all cancer registries across the world (except Maoris) as depicted in the above graphs. However in some populations where the life expectancy of women is higher than men, a higher number of cases are being noted in the cohort of older women (UK 2003 data. Fig1.2, page 19)

1.3.5 Tobacco smoking:

Tobacco is one of our society's most dangerous products. Its consumption in any form is detrimental to health. Nearly half of all who smoke for nearly all their life will

succumb to diseases directly related to it (Doll et al., 1994). This elevated mortality is attributed to cardio-vascular, cerebrovascular and various other forms of arterial diseases, COPD and cancers at various sites (Jacobs et al., 1999) (Table 1.3). Smoking is responsible for nearly 20-30% of all cancers (Tominaga, 1999, 1982) which include cancers of the oral cavity, oro- and hypo-pharynx, nasal cavities and sinuses, larynx, lung, oesophagus, stomach, liver, pancreas, colon and rectum, cervix and myeloid leukaemia.

Table 1.3: Health consequences of tobacco smoking (From Harrison's Principles of internal medicine 15th Edition)

Cancers caused by smoking	**Other potentially fatal illnesses caused**	**Non-fatal illnesses**	**Risk in Pregnancy**	**Protective**
Lung	CAD	PVD	Spontaneous abortion	Parkinson's disease
Upper respiratory sites	COPD	Cataracts	Ectopic pregnancy	
Urinary bladder	CVA	Osteoporosis	Low birth weight	
Pancreas	Pneumonia	Periodontal disease	Limb reduction defects	
Oesophagus	Aortic aneurysm			
Stomach	Pancreatitis (Acute and Chronic)			
Kidney	Diabetes			
Uterine cervix				

The strongest avoidable risk factor in sporadic pancreatic cancer development is tobacco smoking. Tobacco smoking is associated with an increased risk of pancreatic cancer – an overall relative risk of 1.5 to 1.76 in smokers as compared to non-smokers (Andre et al., 1998) (Baghurst et al., 1991). This increased risk falls to near normal

levels in “ever-smokers” only after 15 years of cessation of smoking (Bueno de Mesquita et al., 1991). There is a tobacco dose-related increase in the incidence of pancreatic cancer. About 30% of pancreatic cancers are smoking related (Silverman et al., 1994) (Fuchs et al., 1996) (Iodice et al., 2008).

The risk of pancreatic cancer in smokers is 2.5 to 3.6 times that in non-smokers; the risk increases with greater tobacco use and longer exposure to smoke. Data are limited on the possible roles of intake of coffee, and use of aspirin as contributing factors. Some studies have shown an increased incidence of pancreatic cancer among patients with a history of diabetes or chronic pancreatitis, and there is also evidence, although less conclusive, that obesity (including high-fat, high-cholesterol diet) (Bracci, 2012), and previous cholecystectomy are associated with an increased incidence. Although earlier studies had not suggested a role for alcohol consumption in pancreatic cancer, recent evidence suggests that excessive alcohol intake does increase risk for pancreas cancer (Gupta et al., 2010, Duell, 2012, Lucenteforte et al., 2012). From 2009 to 2012, 3 pooled analyses (Lucenteforte et al., 2012, Michaud et al., 2010, Genkinger et al., 2009) and 1 meta-analysis (Tramacere et al., 2010) have interrogated the role of alcohol and in summary they do suggest that individuals who consume higher levels of alcohol (>30–40 g alcohol/d, or >3 alcoholic drinks/d) may be at an increased risk of pancreatic cancer (Duell, 2012).

1.3.5.1 Tobacco derived carcinogens

Tobacco smoke contains 4000 compounds and 50 carcinogens as compared to 3000 compounds and 30 carcinogens in processed unburnt tobacco (Hecht, 1998). The components of mainstream and side stream smoke are different. Both are harmful. These carcinogens include polynuclear aromatic hydrocarbons (PAH), tobacco specific nitrosamines (TSNA), aromatic amines, aza-arenes, aldehydes, other organic compounds like benzene, inorganic compounds like hydrazine and various metals (Hecht and Hoffmann, 1988). The major classes of carcinogens have been identified as PAH's, TSNA's and aromatic amines (Hecht and Hoffmann, 1988). In the context of pancreatic cancer TSNA's, especially 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) are of utmost importance because they demonstrate organ specificity towards the pancreas and are the only carcinogens to induce pancreatic adenocarcinoma in animal models when given systemically (Rivenson et al., 1988). The most widely used and studied carcinogen model is Syrian gold hamsters intraperiotoneally injected with N-nitrosobis(2-oxopropyl)amine. Other approaches use azaserine in rats and 7,12-dimethylbenzanthracene in mice (Osvaldt et al., 2006) (Ding et al., 2010), however in these methods the carcinogen is directly exposed into the peritoneal cavity or onto the pancreas and is not administered systemically. TSNA's are present in large quantities in both burnt and un-burnt tobacco. Seven compounds have been identified in the family of TSNA's – N′-Nitrosonornicotine (NNN), NNK, NNAL, N′-Nitrosoanatabine (NAT), N′-Nitrosoanabasine (NAB), 4-(methylnitrosamino)-4-(3- pyridyl)-1-butanol (iso-NNAL) and 4- (methylnitrosamino)-4-(3-pyridyl)butyric acid (iso-NNAC). Amongst these NNN, NNK and NNAL are the most powerful carcinogens. Following exposure these carcinogens need to undergo a series of reactions in vivo – uptake, metabolic activation and DNA adduct formation, which subsequently leads to altered growth

kinetics in the target organ and development of neoplasia. NNK is rapidly distributed to most tissues and is rapidly metabolized. NNK and NNAL undergo carbonyl reduction, pyridine N-oxidation and α-hydroxylation. In humans the NNK-NNAL equilibrium favours NNAL. It has been shown that aromatic amines and nitro aromatic hydrocarbons are metabolically activated in the human pancreas (Anderson et al., 1997). It has been demonstrated that cotinine, NNK and its metabolite NNAL are present in the pancreatic juice of smokers (Prokopczyk et al., 2002). This confirms that the pancreas is exposed to TSNA's and that these carcinogens may play a role in carcinogenesis at this site (Schulze et al., 1992). In rodents it has been shown that NNK and NNAL are excreted in the bile in significant concentrations (Schulze et al., 1992). If this is true in humans, it may be the route through which activated carcinogens reach the head of the pancreas (carcinogen containing bile refluxing into the pancreatic duct). This theory is attractive given the established fact that the pancreatic head is the most frequent site for adenocarcinoma (Niedergethmann et al., 2002, Conlon et al., 1996, Iakimov and Zheleva, 1991). A study in rhesus monkeys (n=4), however, found that biliary excretion of the NNK metabolites was significantly less than that predicted from rat experiments. (Meger et al., 1999). Very limited experiments have been performed on this route of TSNA excretion.

1.3.5.2 Tobacco derived carcinogens and pancreatic tumours in animals:

Numerous animal models of pancreas ductal cancer have been developed and these have proved invaluable in elucidating the kinetics of carcinogens within physiological systems and the pathogenesis of ductal cancer of the pancreas. In 1988, Hecht and Hoffman highlighted the important role of tobacco-derived carcinogens in extra-pulmonary cancers including the pancreas in a commentary (Hecht and Hoffmann,

1988). The same commentary also concluded that two of the nicotine-derived nitrosamines, NNK and NNN, are strong carcinogens in laboratory animals and that they could induce tumours both locally and systemically. Analytical and dosimetric studies in animals and humans have confirmed that the magnitude of the total doses of NNK and NNN required to produce cancer in laboratory animals is similar to the total estimated doses which long-term snuff-dippers or heavy smokers are exposed to in their life-time (Hecht and Hoffmann, 1988).

In F-344 rats, NNK and NNAL cause development of not only lung but also pancreatic adenocarcinoma. The doses of carcinogens to body weight needed to cause these neoplasms in rats were similar to lifetime exposure of these chemicals in heavy smokers (40 cigarettes/day). This was the first example of pancreatic tumour induction by a component of tobacco smoke. NNAL appeared to be the proximate pancreatic carcinogen of NNK as it induced more tumours than NNK (Rivenson et al., 1988). They are the only carcinogens to induce pancreatic adenocarcinoma in animal models when given systemically (Rivenson et al., 1988, Hoffmann et al., 1991). Also in F-344 rats there is evidence that following oral NNK administration, there is preferential metabolism of NNK to (S)-NNAL followed by its extensive retention in various target tissues including the pancreas of NNK- orally treated animals (Zhang et al., 2009). Treatment of rats with TSNA's also resulted in the induction of pancreatic acinar cell and ductal cell neoplasms (Pour and Rivenson, 1989).

In rodents it has been shown that NNK and NNAL are excreted in the bile in significant concentrations (Schulze et al., 1992). If this is true in humans, it may be the route through which activated carcinogens reach the head of pancreas (carcinogen containing bile refluxing into the pancreatic duct). This theory is attractive given that the pancreatic

head is the most frequent site for adenocarcinoma (Niedergethmann et al., 2002, Conlon et al., 1996, Iakimov and Zheleva, 1991).

1.3.5.3 Tobacco carcinogens in humans and their relevance to pancreatic cancer:

Most animal studies have been extrapolated to humans and the assumption is that the metabolism and physiological distribution kinetics of tobacco derived carcinogens is similar to that in rats and rodents. However there will be differences in the manner in which our species handles these carcinogens and a few studies have tried to elucidate this by various means. Essentially what has been achieved is to show that the pancreas is exposed to these chemicals and that these metabolites bind to pancreatic ductal cell DNA and result in mutations there and that various phenotypic changes occur there including cancer. However the exact mechanism by which these carcinogens turn a normal ductal cell into a malignant one has not been described.

TSNA's (NNK and NNAL) have been detected in the pancreatic juice of smokers in significantly higher quantities as compared to non-smokers confirming that the pancreas is exposed to these carcinogens (Prokopczyk et al., 2002). It has been shown that aromatic amines and nitro aromatic hydrocarbons are metabolically activated in the human pancreas (Anderson et al., 1997). This confirmed that the pancreas is exposed to TSNA's and that these carcinogens may play a role in carcinogenesis at this site (Schulze et al., 1992).

Although a strong correlation had been suggested between cigarette smoking and pancreatic cancer, studies on pathological changes in the pancreas of smokers are infrequent. A comparative autopsy study (Tomioka et al., 1990) on 73 pancreases obtained from 42 heavy cigarette smokers and 31 non-smoker patients revealed an

increased incidence of pancreatic cancer in smokers than in non-smokers, the difference was statistically not significant. Ductal changes, including mucinous or squamous cell metaplasia and papillary hyperplasia, were found with equal frequencies in both groups of patients and the authors concluded that the type and the incidence of these ductal alterations were not related to smoking but to the age. There were significant limitations of this autopsy study including limited number of the sections of the pancreata examined, as well as exclusion of other important variables, such as alcohol, diet and diabetes weaken the value of this study (Tomioka et al., 1990). Another autopsy study obtained purified DNA from human lung, liver, bladder, pancreas, breast and cervix of 13 men and 6 women and analysed it for DNA adducts using a modification of the 32P post-labelling technique. Relatives were asked to provide information on smoking history for deceased subjects. All tissues examined except the breast had detectable adducts. In lung, bladder and pancreatic tissue a characteristic pattern of adducts was seen which had previously been reported as typical of cigarette-smoke-induced damage; "diagonal reactive zone" (Randerath et al., 1988). Smokers and former smokers tended to have higher adduct levels than non-smokers in the tissues examined but this was only significant for the lung. These results confirmed the finding that cigarette smoking is associated with DNA damage in the lung and suggested that similar damage may be related to tobacco-induced neoplasms of other tissues (Cuzick et al., 1990). TSNA's adducts have been found in the pancreas and the levels have correlated with dose and time related to exposure (Randerath and Randerath, 1993).

1.3.5.4 Epidemiological studies:

There have been numerous case-control and cohort studies from various geographical areas of the world. These have demonstrated increased risk of pancreatic adenocarcinoma in smokers ranging from 1.96 – 5 times that of non-smokers (Appendix

3). The risk increases with increasing exposure in terms of pack-years (Ghadirian et al., 1991, Harnack et al., 1997). The increased risk appears to reduce after 10-15 years of quitting smoking (Boyle et al., 1996). It is variously estimated that approximately 25% (Fuchs et al., 1996), 26% (in Whites) and 29% (in Blacks) (Silverman et al., 1994) of all pancreatic cancers are attributable to tobacco smoking. A recent meta-analysis (Iodice et al., 2008) demonstrated the significant strength of the association between cigarette smoking and pancreas cancer and calculated that the population attributable risk secondary to tobacco use for the malignancy was about 20%.

One of the significant difficulties many epidemiological studies suffer from is that of estimation of tobacco exposure. A questionnaire, which attempts to document the tobacco habit, is commonly used in these studies.

Cumulative exposure based on this method depends upon the method by which the questionnaire was answered – self administered/ administered by trained personnel, patient/next of kin answering the questions, type of questionnaire used etc. The method of administration of the questionnaire can have significant implications on the data obtained (Bowling, 2005, Choi and Pak, 2005), however until a biomarker is identified which can document cumulative exposure the questionnaire method will be the most commonly used.

1.3.6 Environmental factors including industrial exposure to chemicals

Various occupations have been associated with a high risk of pancreatic cancer ranging from – administration and management to leather tanning, rubber workers, petroleum industry and dry cleaning. Most environmental factors which are associated with an increased risk of pancreatic cancer are probably due to exposure to aromatic amines, chlorinated hydrocarbon, silica, cadmium and nickel (Weiderpass et al., 1998).

Section 1.4: Pancreatic carcinogenesis

The primary aetiology of pancreatic ductal adenocarcinoma is poorly understood. Both genetic and environmental factors play a role as shown by various epidemiological, molecular epidemiological and molecular genetic studies. There are genetic disorders in which pancreatic adenocarcinoma is a major feature and familial pancreatic cancer is a well-recognized pathological entity. Over the past 10-15 years extensive work into the development of pancreatic cancer has been carried out. A model of step-wise progression from normal to malignant cells and the molecular alterations involved in these has been suggested (Hruban et al., 2000) (Figure 1.4).

1.4.1 Molecular progression model

Very similar to the adenoma-carcinoma sequence in the colon, neoplastic progression in the pancreatic ducts has been proposed (Hruban et al., 2000, Goggins et al., 2000, Wilentz et al., 2000a, Wilentz et al., 2000b). This progression from a normal pancreatic ductal cell to an infiltrating carcinoma is thought to involve sequential multiple genetic alterations and is pictorially depicted in Figure 1.4. The genetic changes include activating point mutations in K-ras, overexpression of HER-2neu, and inactivation of p16, p53 DPC4 and BRCA2. A gatekeeper gene for the initiation of pancreatic neoplasia has not been identified and the cause of the ignition of change in a normal ductal cell towards neoplasia is under investigation.

The various histological changes in the pancreatic ducts were classified systematically by a Pancreatic Cancer Think-Tank (http://pathology.jhu.edu/pancreas_panin.), which, was sponsored by the National Cancer Institute in 1999 and the term PanIN (pancreatic intra epithelial neoplasia) was introduced.

1.4.1.1 PanIN-1A (Pancreatic Intraepithelial Neoplasia 1-A)

These are flat epithelial lesions composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin. The nuclei are small and round to oval in shape. When oval the nuclei are oriented perpendicular to the basement membrane. It is recognized that there is considerable histologic overlap between non-neoplastic flat hyperplastic lesions and flat neoplastic lesions without atypia. Therefore, some may choose to designate these lesions with the modifier lesion ("PanIN/[L]-1A") to reflect the fact that the neoplastic nature of many cases of PanIn-1A has not been established.

1.4.1.2 PanIN-1B (Pancreatic Intraepithelial Neoplasia 1-B)

These epithelial lesions have a papillary, micro papillary or basally pseudo-stratified architecture, but are otherwise identical to PanIN-1A.

1.4.1.3 PanIN-2 (Pancreatic Intraepithelial Neoplasia 2)

Architecturally these mucinous epithelial lesions may be flat or papillary. Cytologically, by definition, these lesions must have some nuclear abnormalities. These abnormalities may include some loss of polarity, nuclear crowding, enlarged nuclei, pseudo-stratification and hyperchromatism. These nuclear abnormalities fall short of those seen in PanIN-3. Mitoses are rare, but when present are non-luminal (not apical) and not atypical. True cribriforming luminal necrosis and marked cytologic abnormalities are generally not seen, and when present should suggest the diagnosis of PanIN-3.

1.4.1.4 PanIN-3: (Pancreatic Intraepithelial Neoplasia 3)

Architecturally these lesions are usually papillary or micro papillary, however, they may rarely be flat. True cribriforming, budding off of small clusters of epithelial cells into the lumen and luminal necrosis all suggests the diagnosis of PanIN-3. Cytologically,

these lesions are characterized by a loss of nuclear polarity, dystrophic goblet cells (goblet cells with nuclei oriented towards the lumen and mucinous cytoplasm oriented toward the basement membrane), mitoses which may occasionally be abnormal, nuclear irregularities and prominent (macro) nucleoli.

Normal duct epithelium is proposed to progresses to infiltrating cancer (*left* to *right in Figure 1.4*) (Hruban et al., 2000) through a series of histologically defined precursors (PanINs). The over expression of HER-2/*neu* and point mutations in the K-*ras* gene occur early, inactivation of the *p16* gene at an intermediate stage, and the inactivation of *p53, DPC4,* and *BRCA2* occur relatively late (Table 1.4).

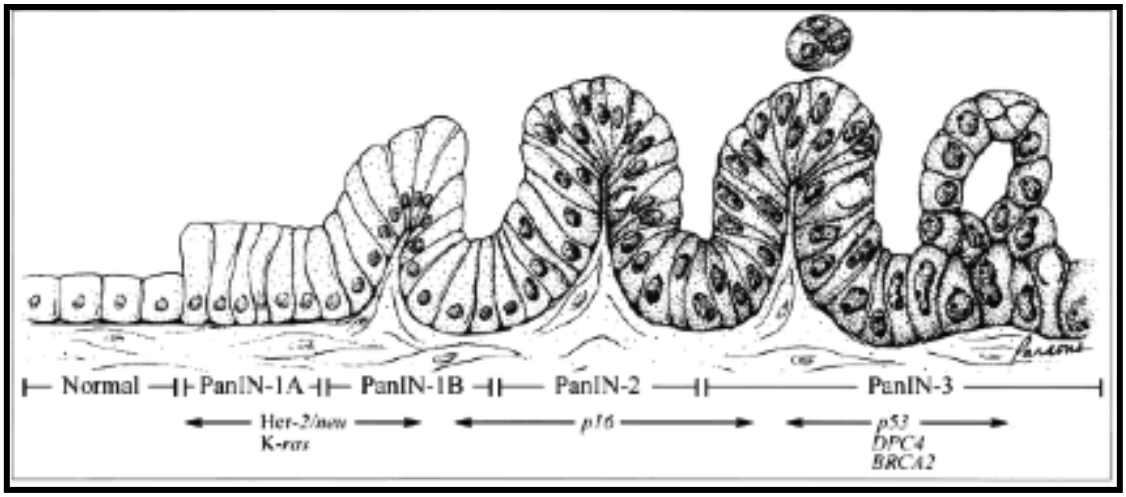

Figure 1.4 Progression of precursor lesions to invasive cancer (Hruban et al., 2000)

Table 1.4: Frequencies of genetic alterations in infiltrating ductal adenocarcinoma and its precursors

Gene	Normal	PanIN1A	PanIN1B	PanIN2	PanIN3	Ca	Selected References
K-ras	0-15%	35%	86%	92%	100%	90%	(Moskaluk et al., 1997, Caldas et al., 1994) (Tada et al., 1996)
Her-2neu	5%	82%	86%	92%	100%	69%	(Day et al., 1996)
P16	0	24%	19%	55%	71%	95%	(Wilentz et al., 1998)
P53	0	0	0	NA	21	75	(DiGiuseppe et al., 1994)
DPC54	0	0	0	0	31	55	(Wilentz et al., 2000b)
BRCA2	0	0	0	0	0	7	(Goggins et al., 1996)

1.4.2 Neoplastic pancreatic cysts:

Whilst discussing pancreatic carcinogenesis, it is important to discuss the other precancerous/precursor lesions associated with it, i.e. Intraductal papillary mucinous adenoma (IPMN). Cystic lesions are increasingly detected given the growing availability and use of cross-sectional imaging techniques. The prevalence of these lesions is difficult to estimate, as the majority are asymptomatic and are detected when imaging is performed for unrelated symptoms. It is estimated that the prevalence of cystic lesions of the pancreas is about 20% in patients undergoing imaging for non-

pancreatic illnesses (Zhang et al., 2002) and 25% in an autopsy study from Japan (Kimura et al., 1995). Out of these 10-15% are cystic neoplasm's whilst the remainder are pseudocysts (Warshaw and Rutledge, 1987). Table 1.5 depicts the clinic-pathological classification of neoplastic pancreatic cysts

Table 1.5: Classification of neoplastic pancreatic cysts (Adapted from Hutchins and Draganov, Surg Clinic N Am April 2010)

Serous tumours	Serous cystic tumours	Serous cystadenoma
		Serous cystadenocarcinoma (Very rare)
Mucinous cystic tumours	Mucinous cystic tumours	Mucinous cystadenoma
		Mucinous cystadenoma with moderate dysplasia
		Mucinous cystadenocarcinoma
		Non-infiltrating
		Infiltrating
	IPMN	Intraductal papillary mucinous adenoma
		IPMN with moderate dysplasia
		Intraductal papillary mucinous carcinoma
		Non-infiltrating
		Infiltrating
Solid pseudopapillary tumours	Solid pseudopapillary tumours	

After an inflammatory cyst (pseudocyst) has been excluded the distinction between the neoplastic cysts is important to make as the malignant potential associated with the lesion depends upon this.

1.4.2.1 Mucinous cystic neoplasms:

The 2 main types of cystic lesions seen in the pancreas are the serous and mucinous cystadenomas (MCN). The serous lesions are largely benign lesions, which are managed non-surgically. The important distinction in these classes of pancreas tumours is the one between MCN and intraductal papillary mucinous neoplasms (IPMN). This depends on clinical and pathologic factors. Clinically, main duct IPMN with malignant transformation is seen archetypically in the head of the pancreas in an elderly male whereas side branch IPMN are not sex-specific and are distributed throughout the pancreas. Main duct IPMN has a characteristic grape-like clustered appearance of individual cysts on imaging whilst MCN has the appearance of multiple cysts within cysts. The lesion communicates with the pancreatic duct. MCN is typically seen in a middle-aged female and is located in the body and/tail of the pancreas. Pathologically the best differentiating feature (from IPMN) is the presence of an ovarian stroma in MCN (Volkan Adsay, 2007) and it is suspected that MCN arise from ovarian rests within the pancreas.

Of the MCN 6-36% are malignant (Reddy et al., 2004) and result in mucinous cystadenocarcinoma. Various features have been described which predict malignancy – thickened and or irregular cyst wall, papillary projections, mixed solid and cystic components, symptomatic lesions, tumours with radiologically visualised calcification, hyper-vascularity and major vascular involvement.

1.4.2.2 Intraductal papillary mucinous neoplasms (IPMN):

Intraductal papillary mucinous neoplasms are an increasingly recognised group of neoplasms, which differ from mucinous cystic tumours by their diffuse/multi focal nature. Therefore they are considered to represent a "field change" within the pancreatic duct system (Sohn et al., 2004). Based on extent of involvement of the duct system they are classified into – main duct type, branch duct type and mixed. They are pre-malignant and main duct-IPMN is associated with malignancy in 57 – 92%, branch duct -IPMN with 6-46% of and mixed-IPMN is associated with malignancy in 35-40% resected cases (Sohn et al., 2004, Schnelldorfer et al., 2008, Salvia et al., 2004). Depending upon degree of dysplasia increasing grades are classified – adenoma, borderline dysplasia, and carcinoma. IPMN's with severe dysplasia are designated as carcinoma even in the absence of invasion. They are presumed to progress from adenoma to invasive cancer in a manner similar to the colon adenoma-carcinoma sequence – varying degrees of dysplasia exist within one single tumour and older patients with IPMN have an increased risk of harbouring cancer within them (Hruban et al., 2005).

The diagnosis of IPMN is made on triple phase pancreas CT and or MRCP, both of which demonstrate the pancreatic ductal abnormality accurately. MR is more accurate in demonstrating the communication between BD-IPMN (branch duct IPMN) and the ductal system. Features of malignancy – bulging papilla, presence of a solid component, local invasion, mural nodules, diffuse/multifocal involvement of the duct, calcification/attenuating intraluminal content are all equally well seen on CT and MR (Ogawa et al., 2008, Fukukura et al., 2003). IPMN is easily differentiated from mucinous cystic neoplasm, as MCN is a unifocal, round cystic lesion, which does not communicate with the PD.

Endoscopic Ultrasound (EUS) with Fine-needle aspiration (FNA) is used to confirm the diagnosis and differentiate it from other cystic neoplasms (Kobayashi et al., 2012, Ohno et al., 2012). It can visualize the ductal dilation, papillary projections and mural nodules that are typical of IPMN. The main advantage of EUS is the ability to perform an FNA at which cyst fluid can be macroscopically, microscopically, cytologically and biochemically examined. Peroral pancreatoscopy and intraductal ultrasound are newer modalities, which may be useful in the future.

Carcino-Embryonic Antigen (CEA) greater than 192 ng/mL (Brugge et al., 2004), papillary fragments, parachromatin clearing, atypical clusters, hypercellularity and necrosis suggest malignancy (Michaels et al., 2006). Other factors which predict malignancy in IPMN's include type (main duct type has higher risk), larger tumour diameter (more than 30 mm), proximal location, involvement of a dilated main pancreatic duct which is more than 7 mm, presence of mural nodules, protruding lesions in dilated side branch ducts, thickened cyst walls, patulous papilla with mucin seepage from it and a raised CA 19-9 level, older age, presence of jaundice, diabetes and episodes of pancreatitis (Garcea et al., 2008b, Salvia et al., 2004, Fujino et al., 2007). IPMN malignancies grow less aggressively, have lower nodal spread with less incidence of perineural and vascular invasion as compared to ductal adenocarcinoma (Sohn et al., 2004).

Management can vary from observation to pancreatic resection. The risk of malignancy dictates the course of action. Main and mixed IPMN in fit individuals are considered for resection, given the significant risk of malignancy. In BD-IPMN the presence or absence of factors which suggest/refute malignancy are crucial in the decision making process. The International Association of Pancreatology (IAP) guidelines (Sendai

guidelines) (Tanaka et al., 2006) are an important source of guidance in the management of these complex lesions.

Section 1.5: DNA Repair

DNA undergoes damage due to both endogenous and exogenous mutagens. It is most essential that the damaged DNA is repaired to avoid unplanned apoptosis, changes in cellular differentiation and proliferation and thereby the development of neoplasms (Barnes and Lindahl, 2004). Genetic syndromes, which result in cancer due to a defect of the DNA repair mechanisms are described: notably Xeroderma pigmentosa (Kraemer et al., 1994), Lynch syndrome (Hassen et al., 2012) etc. Various mechanisms exist in both mammalian and lower forms of life (including bacteria i.e. prokaryotes) and have been conserved through evolution to recognize and repair the damage caused to DNA (Taylor and Lehmann, 1998). At least 4 different pathways have been identified and they repair different types of damage. Each type of repair involves various molecules and enzymes, which act in a series of steps. The type of repair initiated depends on the nature of the mutagen.

1.5.1 Base excision repair (BER):

Base excision repair recognizes and removes small DNA lesions which are typically oxidised or reduced bases, fragmented or non-bulky adducts and those produced by methylating agents (Fig 1.5). The single damaged base is removed by a specific DNA glycosylase and replaced by an endonuclease (figure below). The molecules involved are APEX/APE (apurinic/apyrimidinic endonuclease), DNA polymerase-β and XRCC1 (X-ray complementing cofactor 1). BER plays an important role in preventing mutations associated with a common product of oxidative damage to DNA, 8-oxoguanine. X-Ray Repair Cross-Complementing Group 1 (XRCC1), located on 19q13.2, is a polymorphic BER gene that has been the most extensively examined in molecular epidemiologic studies of the risk of various cancers (Hung et al., 2005c). The

XRCC1 protein is essential for mammalian viability (Thompson et al., 1990) and its deficiency in mice results in embryonic lethality (Thompson and West, 2000), and XRCC1 is required for the efficient repair of single- strand breaks and damaged bases in DNA. XRCC1 has no known enzymatic activity but is thought to act as a scaffold protein for both single-strand break repair and base excision repair activities (Lindahl and Wood, 1999). XRCC1 has been shown to physically interact with DNA polymerase b, polyadenosine diphosphate-ribose polymerases 1 and 2, APE1/APEX1, OGG1, and proliferating cell nuclear antigen, poly ADP-ribose polymerase (PARP), DNA ligase III, DNA polymerase-b etc (Caldecott, 2003, Fan et al., 2004). The functional significance of XRCC1 280 has not been established, although, the 280His allele was suggested in a small study (n = 80) to be associated with higher bleomycin sensitivity (Tuimala et al., 2002).

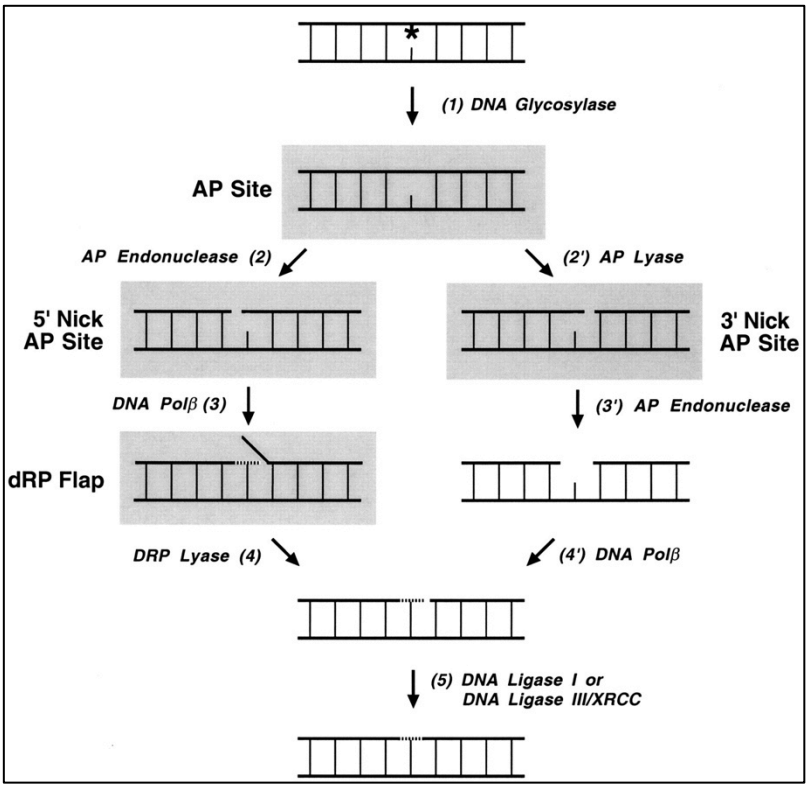

Fig 1.5: Base Excision Repair

A DNA glycosylase such as OGG1 (Human 8-oxoguanine DNA glycosylase 1) initiates BER by releasing the altered base (in the case of OGG1 it is 8-oxo-guanine). There are about 11 such glycosylases in mammals (Barnes and Lindahl, 2004) each of which performs a specific function. Some glycosylases (bifunctional glycosylases) have an associated apurinic/apyrimidinic lyase activity and further catalyse the cleavage of the sugar-phosphate chain and excise the abasic site. Thus a single nucleotide gap results, which is filled by DNA polymerase β and the chain is sealed by DNA ligase 3/XRCC 1 complex. Certain glycosylases have no associated lyase activity and when such enzymes initiate BER, the subsequent step is carried out by a dedicated apurinic/apyrimidinic endonuclease (APE1/APEX1). This is called short-patch BER as one single nucleotide is replaced (Dianov et al., 2003). Long-patch repair is a variant of BER which results in the replacement of several nucleotides – when the sugar-phosphate residue at the abasic site is resistant to cleavage, a few more nucleotides are added to the 3' end by a polymerase and a flap is generated containing the 5' sugar phosphate. This flap is removed by flap endonuclease 1 (FEN1) and DNA ligase completes the repair (Dianov et al., 2003).

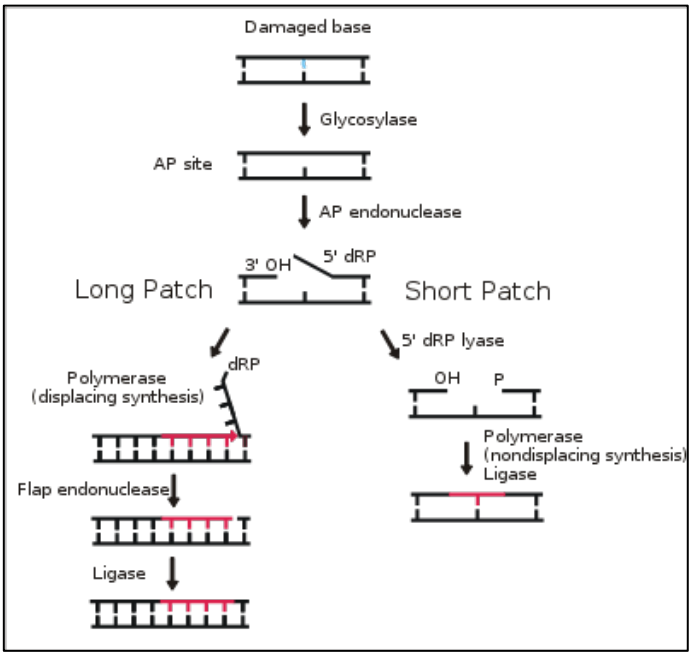

Fig 1.6: BER - Long and short patch (Dianov et al., 2003)

Sequence variants in DNA repair genes have been thought to modulate DNA repair capacity and thereby are suggested to be associated with altered cancer risk. However the results from epidemiological studies have been inconsistent. This is possibly due to (Hung et al., 2005c)

- Low statistical power for detecting a moderate effect (i.e false negative results)
- False-positive results
- Heterogeneous study populations
- Failure to consider effect modifications such as environmental exposures
- Publication bias – including the absence of publication of negative studies

There is therefore a need for studies to address the above issues. It is also important for large consortiums to undertake such molecular epidemiological studies, which will enable nearly all of the above problems to be overcome.

There has been a paucity of studies investigating the role of DNA repair in pancreatic carcinogenesis, though there are indications that DNA repair plays an important role in development of pancreatic cancer

1.5.2 Nucleotide Excision Repair (NER):

Nucleotide excision repair repairs bulky lesions such as pyrimidine dimers, large chemical adducts (PAH adducts) and cross-links between DNA. Photo-damage by UV light and radiation induced damage is also repaired by NER. This pathway involves at least 4 steps (Figure 1.7).

- Damage recognition by various proteins including XPC
- Unwinding of the DNA by TFIIH complex (which includes XPD)
- Removal of the damaged single strand which involves ERCC1 and XPF complex
- Synthesis of a new oligo-nucleotide chain by DNA polymerases

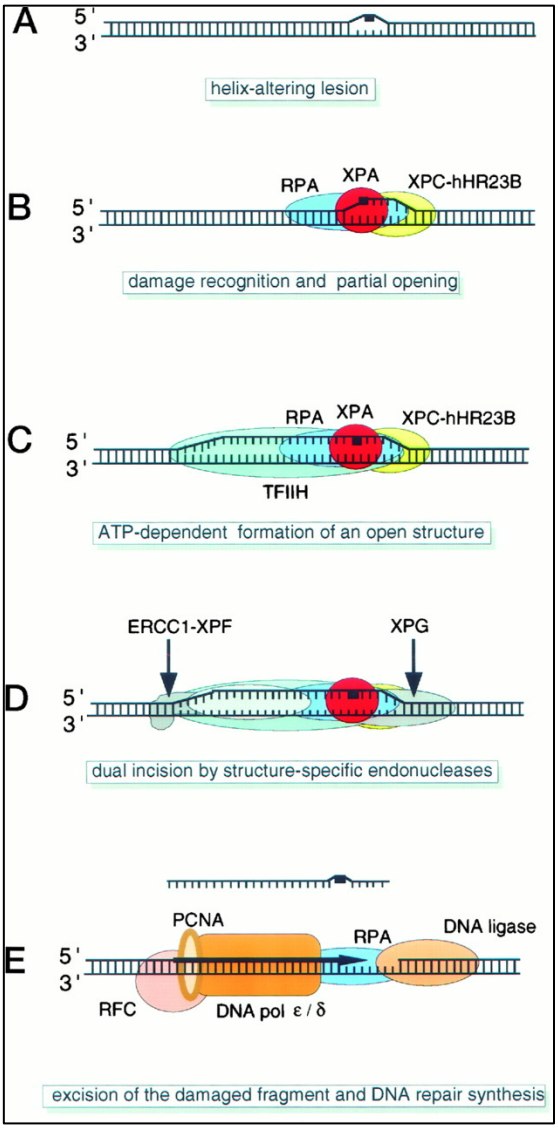

Fig 1.7: Nucleotide Excision Repair ((Wood, 1997)

1.5.3 Double Strand Break Repair (DSB):

Double strand break repair can be produced endogenously by replication errors and by exogenous agents such as radiation. The repair of such lesions is much more complex than other types of damage, mainly because there is no undamaged template with which to deduce the damaged strand. Two pathways of DSB repair are known homologous recombination (Fig 1.8) and non-homologous end joining. Homologous recombination involves resection of the ends of the damages DNA, invasion of the undamaged strand of the double helix by the 3'ends of the newly resected DNA strand, extension by polymerases and exchange of strands resulting in 2 intact molecules (Khanna and

Jackson, 2001). This involves the products of various genes including BRCA1, BRCA2 and XRCC3. Non-homologous end joining involves direct ligation of the DNA ends and also involves numerous molecules (Khanna and Jackson, 2001).

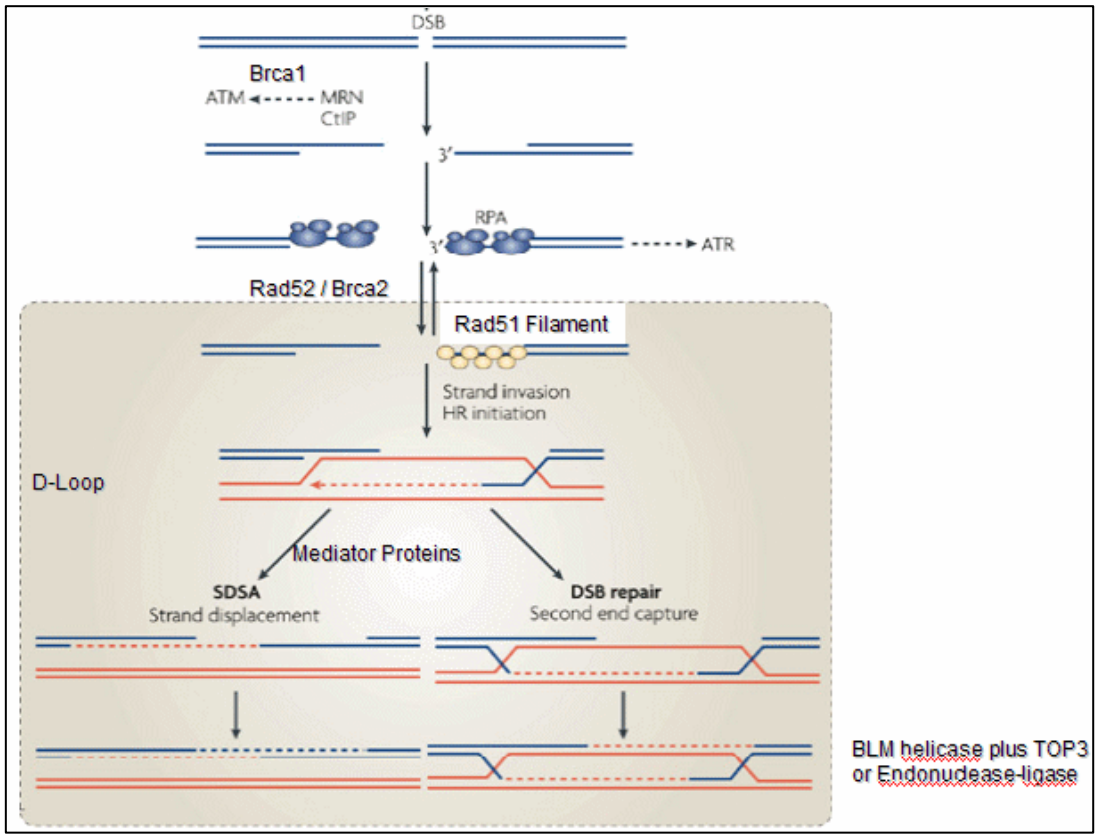

Fig 1.8 DSB repair Homologous recombination

1.5.4 Mis-Match Reapir (MMR):

The mismatch repair (MMR) mechanism (Fig 1.9) is a highly conserved group of proteins from bacteria to humans) that function in maintenance of the genome and avoid mutation. They correct replication errors caused by DNA polymerase errors. These are base-base mismatches or insertion-deletion mismatches (Aquilina and Bignami, 2001) and DNA loops that contain repeated sequences (Parsons et al., 1995). These mismatches arise endogenously during replication and homologous recombination. Mismatches can also result from exogenous DNA damage. MMR proteins are also

involved in regulation of meiotic chromosomal pairing and immunoglobin class switching. There is also recent evidence that 8-oxo-guanine, which is a major lesion resulting from oxidative damage to DNA, mismatches to Adenine. This 8-GO:A mismatch is repaired by MMR in E.Coli (Lu et al., 2001). The genes involved in MMR are MSH-2, MSH-3, MSH-6, hMLH1 (Kolodner and Marsischky, 1999). The region of chromosome 3p21 has recently been shown to be sensitive to tobacco smoking induced damage and is also the locus for hMLH1 and loss of heterozygosity at this region has been demonstrated (Hirao et al., 2001).

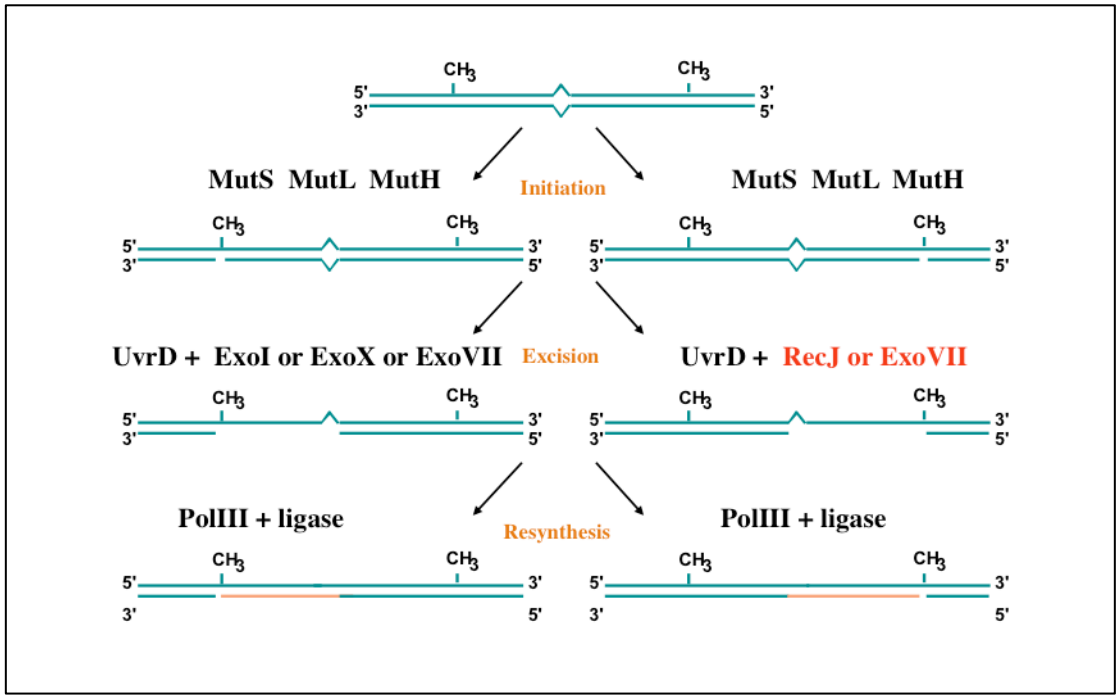

Fig 1.9 Mechanism of Mis-match repair

Cells deficient in MMR exhibit a mutator phenotype in which spontaneous mutation rates are highly increased in many genes due to uncorrected errors in DNA replication. When the DNA template strand becomes disassociated from the strand that is being synthesized, repeated sequences may not reassociate correctly, resulting in loops and slipped mispairing. Uncorrected loops result in deletions and insertions in the repeat region. Deficient mismatch repair, due to a mutation in the MMR genes would lead to

no or reduced efficiency of repair of the mis-matched bases. Tumours with this type of phenotype display microsatellite instability. A panel of 5 markers - BAT-25, BAT-26, D2S123, D5S346 and D17S250, called the Bethesda panel of markers are used to distinguish high frequency of microsatellite instability (MSI-H – 2 or more unstable markers) from MSI-L [(low) – 1 unstable marker] and MSS (no unstable marker) (Boland et al., 1998). The classical disease of MMR deficiency is HNPCC – hereditary non-polyposis colorectal carcinoma, which is secondary to a heritable mutation in MSH-2 or MLH-1. The colo-rectal cancers in HNPCC are universally MSI-H. MSI-H is also seen in 15% of sporadic colonic cancers, endometrial carcinoma, pancreatic adenocarcinoma, prostatic adenocarcinoma, small cell lung carcinoma and renal cell carcinoma (Toft and Arends, 1998).

1.5.5 Diseases caused by inherited defects in DNA repair genes

Since DNA repair systems play such a crucial role in the maintenance of the genome, deficiency of these repair mechanisms can have significant pathological consequences. A number of diseases are associated with specific defects in DNA repair as detailed in the table 1.6 below.

Table 1.6: Inherited defects in DNA repair mechanisms and consequent illness

Disease	DNA repair defect	Phenotype	Type of genomic instability
Xeroderma pigmentosa	NER	Cancer	Mutations
Trichothiodystrophy	NER/TCR	Brittle hair, developmental defects	Mutations
Cockayne Syndrome	TCR/BER	Neurological defects, Premature ageing	Mutations
Bloom Syndrome	HR	Cancer	Chromosomal aberrations
Werner Syndrome	HR/NHEJ	Cancer, Premature ageing	Chromosomal aberrations
HNPCC	MMR	Cancer	Mutations
Ataxia telangiectasia	unknown	Neurological defects	Chromosomal aberrations
Hereditary breast cancer	BRCA1 & 2 play a role in DNA repair	Familial breast cancer	Mutations
Fanconi anaemia	unknown. Possible HR/Inter strand cross link repair	Cytopenia, short stature, hypersensitivity to UV light	unknown

1.5.6 Cancer due to DNA repair gene mutations

At least 3 cancer causing syndromes occurring due to mutations in the DNA repair genes have been described

Xeroderma pigmentosa – Mutation in NER genes leads to a high incidence of basal cell carcinoma

HNPCC – Mutation in the MMR genes leads to hereditary colon cancer and also an increased risk of other cancers including pancreatic cancer

Hereditary breast cancer – BRCA 1 and BRCA 2 gene mutations cause familial breast cancer and also predispose to other cancers in the affected families, which includes pancreatic cancer. These genes have been shown to play a critical role in DNA repair (Sharan et al., 1997). The BRCA2 protein is able to bind to the human homologue of the yeast RAD51 protein. In yeast this protein participates in the DNA repair of double strand break and recombination.

The database of DNA repair genes is not yet complete and new members will continue to be identified.

Section 1.6: Inter-individual variation in risk for carcinogenesis

Cancer is a complex disease and results from the accumulation of genetic changes, therefore could be considered primarily to be a genetic disease. Accumulation of DNA damage results in disruption of normal cellular function, cellular differentiation and proliferation resulting in the development of an abnormal clone of cells, which undergo expansion and form a neoplasm. Indeed some cancers are purely genetic ie the result of a genetic abnormality (HNPCC) whilst the majority are a result of some external carcinogen (chemical, viral etc). In non-familial carcinogenesis it is a well-established fact that individuals vary in their susceptibility to the development of cancer. Carcinogens enter the human body from a variety of sources, but most need to undergo metabolic activation before they can damage DNA. This ability of the carcinogens is balanced by multiple protective functions within the human body and these include – carcinogen detoxification, DNA repair and programmed cell death of irreparably damaged cells. All these mechanisms exhibit wide inter-individual variation and thus are the source of the wide variation in cancer risk (Fig 1.10) (Lai and Shields, 1999). The role of gene-environment interaction is of utmost importance in the understanding of carcinogenesis in specific organs and therefore of identification of high-risk individuals.

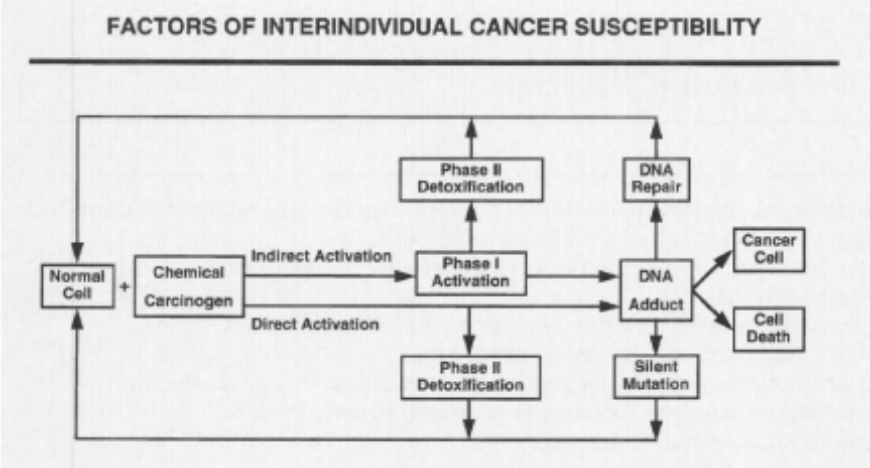

Fig 1.10: The role of inter-individual variation in human carcinogenesis **(Lai and Shields, 1999)**

Epidemiological studies have demonstrated that cancer risks vary in different populations, however it is remarkable that only certain individuals within the same population who are exposed to the same amount of carcinogen develop a particular neoplasm whilst others develop an other illness related to that chemical or do not develop any illness at all. These differences are most likely to be due to inherited traits as discussed in a 1989 commentary (Harris, 1989).

Most of these inter individual differences in cancer susceptibility are thought to be due to variations in the ability to metabolise carcinogens and excrete them from the human body (Phase 1 and 2 enzymes) and also to differing capacities for DNA repair (Bartsch and Hietanen, 1996, Collins, 1998). Some of these differences are secondary to variations in the coding genetic sequence of the genes for the enzymes and proteins involved in these functions. Some genetic variants result in complete absence of a gene whilst others result in the presence of an enzyme/protein with reduced activity. If a genetic variant occurs in >1% of the population then it is considered to be a genetic polymorphism (Lai and Shields, 1999). A single base change in the gene may result in a different amino acid sequence, which might substantially affect activity of the gene

product. These single base changes are called Single Nucleotide Polymorphisms (SNP's). They are common and are the subject of intense investigation.

Apart from a few direct acting carcinogens most need to be metabolically activated before they can exert their harmful action. This metabolism is governed by natural processes, which exist to rid the body of foreign compounds. Phase 1 enzymes convert inert chemicals into electrophilic intermediates by oxidation reactions. Phase 2 enzymes remove the activated intermediates by conjugation with carriers like glutathione. Various studies of polymorphisms of phase 1 and 2 enzymes have been performed and are not detailed here. Base excision repair is an important mechanism which repairs damage caused to DNA as detailed earlier *(vide supra)* and the SNP's in this system have been the subject of extensive investigation and are discussed below.

1.6.1 BER gene variants and their role in development of malignancy

1.6.1.1 hOGG1

The OGG1 gene is located on chromosome 3p26.2. This is a region that demonstrates loss of heterozygosity (LOH) in several human cancers (Shinmura and Yokota, 2001, Kohno et al., 1998). 8-oxoguanine and its tautomer 8-hydroxyguanine are one of the most mutagenic lesions occurring due to base damage by reactive oxygen species.

8-oxoguanine base pairs with adenine and causes G:C $\rightarrow$ T:A transversions in repair deficient bacteria and yeast (Shinmura and Yokota, 2001). At least 20 validated sequence variants have been described and a C $\rightarrow$ G sequence variant leading to an amino acid change from Serine to Cysteine at codon 326 has been most extensively studied – dbSNP no: rs1052133. Various studies have examined the association between genotype variants of OGGI and enzyme activity both in vivo and in vitro and results have been inconsistent (Weiss et al., 2005a). However one paper suggested a

seven-fold higher activity for repairing 8-oxoguanine by 326Ser containing OGG1 to that demonstrated by the 326Cys genotype (Kohno et al., 1998).

From a recent extensive review by Hung et al (Hung et al., 2005c) published in October 2005, the summary Odds Ratio (OR) for lung cancer was 1.09 [95% (CI) 0.86-1.40] for Ser/Cys, and 1.37(CI 1.02-1.82) for Cys/Cys. One outlying study (Park et al., 2004) was removed from the analysis when it was apparent that it was contributing most to the heterogeneity of the data, it was still apparent that there was an increased risk of lung cancer for Cys/Cys carriers with an OR of 1.24 (95% CI 1.01-1.53). This was based on analysis of 3253 cases and 3371 controls from 7 studies. Five studies of upper aero-digestive tract cancers were identified. One study each was excluded due to a very large variance (Elahi et al., 2002) and heterogeneity (Xing et al., 2001). The OGG1 Ser326Cys polymorphism is slightly more prevalent among Asians (39.4–60.6 percent carry the heterozygous variant; 13.4–38.2 percent carry the homozygous variant) than among persons of European descent (23.0–41.0 percent heterozygotes, 1.8–8.6 percent homozygotes). The authors (Hung et al., 2005c) calculated summary odds ration for Asian and Caucasian populations which were 1.16 (95% CI 0.98-1.40) and 1.15 (95% CI 0.90-1.46) respectively.

The summary OR for Asp148Gln APE1/APEX1 was 0.94 (95% CI 0.77-1.14), based on three studies reported until Feb 2005 (Hung et al., 2005c). One study in patients with oesophageal cancer has not demonstrated an association between the Asp148Glu allele of APE/APEX1 and cancer risk (Hao et al., 2004). Recently studies in patients with prostate (Chen et al., 2006) and bladder cancer (Terry et al., 2006) have been reported and effect modification in smokers has been demonstrated in both studies though an increased risk with the variant allele of APE1/APEX1 was not found.

1.6.1.2 XRCC1 Arg194Trp

There was no association between risk of lung cancer and upper aerodigestive tract cancer and this genotype of XRCC1 (Hung et al., 2005a). But when data on all tobacco related cancers from 16 studies were pooled and analysed (4895 cases and 5977 controls) the 194Trp allele was associated with decreased risk – OR 0.86 (95% CI 0.77-0.95). There appeared to be a protective effect among ever-smokers [OR 0.77(95% CI 0.78-1.03)]. No ethnic differences were noted in the risk.

1.6.1.3 XRCC1 Arg280His

No association between this genotype and risk of lung (3 studies), upper aerodigestive (3 studies) or all tobacco related cancers (8 studies) combined were found in a recent meta-analysis (Hung et al., 2005c).

1.6.1.4 XRCC1 Arg399Gln

Thirteen studies which included 6129 cases and 6895 controls did not reveal an association between the 399Gln allele and risk of lung cancer, breast cancer (11 studies) or skin cancer (3 studies) (Hung et al., 2005c). There was a non-significant decreased risk of upper aero-digestive cancers with an OR of 0.88 (95% CI 0.78-1.01). This pooled analysis included 7 studies, on other study(Yu et al., 2004b) was excluded from this review due to heterogeneity. Arg/Gln or Gln/Gln was associated with an summary OR of 1.12(95% CI 0.95-1.33) for risk of bladder cancer, on pooling data from 4 studies with one study exclusion (Matullo et al., 2001a). With regard to the effect of tobacco smoking and risk of tobacco related cancers, the 399Gln allele appeared to increase risk for light smokers [OR 1.20 (95%CI 1.03 – 1.40)], while decreasing the risk for heavy

smokers [OR 0.81(95% CI 0.64 – 1.04)]. The decreased risk was more pronounced with the Gln/Gln genotype [OR 0.71(95% CI 0.51-0.99)].

1.6.1.5 Summary

To summarize an increased risk of lung cancer with the OGG1 Cys/Cys genotype is found. This is consistent with experimental evidence that this gene product exhibits decreased BER activity(Kohno et al., 1998, Dhenaut et al., 2000). The XRCC1 194Trp is protective against risk for tobacco related cancers. This is in keeping with the lower mutagen sensitivity which has been demonstrated with this genotype (Wang et al., 2003). It is to be noted that this effect is seen only in heterozygotes but not in homozygotes. There is evidence for effect modification of the XRCC1 399Gln allele by tobacco smoking. This allele is associated with higher mutagen sensitivity (Wang et al., 2003) and with non-significantly elevated levels of DNA adducts in never smokers and lower adduct levels amongst current smokers (Matullo et al., 2001b). An increased risk of tobacco related cancers is seen amongst light smokers, which is compatible with in vitro studies. The converse of decreased risk among heavy smokers has not been demonstrated epidemiologically. This could be explained by the increased levels of DNA damage resulting from heavy smoking leading to increased apoptosis at cell division and this manifesting as a reduced risk of exposure induced cancer (Nelson et al., 2002). An alternative explanation is that increased tobacco exposure might induce DNA repair capacity in response to DNA damage. This has experimental support in the form of lower chromosome breaks in healthy heavy smokers (Wang et al., 2003). Lower levels of 8-oxoguanine in lymphocytes of smokers as compared to non-smokers provide additional support for this theory (van Zeeland et al., 1999, Besaratinia et al., 2001).

The etiologies of the various molecular alterations involved in pancreatic carcinogenesis are poorly understood. The contributions of genotoxic injury and poor repair of these sites of genomic damage to the development of cancer have not been quantified. Recently it has been shown that codon 12 of human K-Ras may be the preferential "hotspot" for DNA damage by tobacco-derived carcinogens and that poor repair of the carcinogen-DNA adduct at this site may play an important role in initiation of neoplasia (Feng et al., 2002). Tobacco-derived carcinogens play a role in at least one-third of pancreatic cancers. In spite of this very few studies on the role of DNA repair in pancreatic carcinogenesis have been reported. There has been work done on the genetic polymorphisms of carcinogen metabolizing enzymes, which have tried to stratify the varying risks of developing pancreatic cancer in relation to the presence of polymorphisms of the carcinogen metabolizing enzyme systems (Ockenga et al., 2003, Duell et al., 2002a). A population based study, concluded that the combination of heavy smoking and a deletion polymorphism in Glutathione S-Transferase T1 genotype, specifically the presence of the null-genotype and heavy smoking was associated with an increased risk of pancreatic cancer among Caucasians, with the association being possibly stronger in women than men. The same group of scientists recently published another study that analysed the polymorphism in a base excision repair (BER) protein – XRCC1 (X-ray repair cross complimenting group 1) which plays a role in the repair of DNA strand breaks and DNA base damage from a wide spectrum of chemicals, one of which is tobacco smoke (Duell et al., 2002b). This study suggested that the presence of the XRCC1 allele in smokers was a potentially important determinant of susceptibility to pancreatic cancer.

Increasing age is the strongest risk factor for pancreatic cancer and DNA repair decreases with age. It is established that the bulk of DNA repair capacity decreases with age (Bohr and Anson, 1995, Kruk et al., 1995). A study by Wei (Grossman and Wei,

1995) reported a 1% decrease in DNA repair capacity with advancing age. Tobacco smoking has the strongest life-style association with pancreas adenocarcinoma. Studies have recently demonstrated that a sub-optimal DNA repair capacity increases the risk of non-small cell lung cancer associated with smoking (Shen et al., 2003, Gackowski et al., 2003). Similar results have been demonstrated for other tobacco-induced cancers. This evidence makes it attractive to speculate that poor DNA repair plays a major role in the development of pancreatic cancer.

1.6.2 DNA repair, pancreatic adenocarcinoma & need for further studies

Numerous lines of evidence exist in support of a significant role for altered DNA repair in the development of pancreatic cancer. The aetiologies of the various molecular alterations involved in pancreatic carcinogenesis are poorly understood and the contributions of genotoxic injury and poor repair of the sites of genomic damage to the development of cancer have not been quantified. It is pertinent to recall that nearly all pancreatic cancers are associated with a mutant K-ras very early in their development (vide supra). Increasing age is the strongest risk factor for pancreatic cancer and DNA repair decreases with age (Bohr and Anson, 1995, Kruk et al., 1995). Studies have recently demonstrated that a sub-optimal DNA repair capacity increases the risk of non-small cell lung cancer associated with smoking (Shen et al., 2003, Gackowski et al., 2003). Similar results have been demonstrated for other tobacco induced cancers in both animals (Russo et al., 2004) and humans (Hung et al., 2005c).

It is therefore attractive to speculate that poor DNA repair plays a major role in the development of pancreatic cancer and therefore to investigate gene and gene variants involved in DNA repair mechanisms and their interaction with known environmental risk factors in an attempt to define susceptibility groups for the development of

pancreatic cancer. Identification of high-risk groups in this manner may enable targeted screening which could result in earlier diagnoses and improved outcome. Up to the present, few studies concerned with polymorphisms affecting DNA repair genes have been published. The most widely studied polymorphism has been the Arg399Gln polymorphism in XRCC1, which is involved, in base-excision repair. This polymorphism was not a risk factor for pancreatic cancer when cases and controls were compared directly. However, there was evidence for interaction between XRCC1 and smoking and also with a double null GSTM1/T1 genotype (Duell et al., 2002b). In a more recent study which did not genotype for metabolic polymorphisms, XRCC1Arg194Trp was found to interact with polymorphisms in two other repair genes, Asp148Glu in Ape1 and Leu84Phe in MGMT (Jiao et al., 2006a). There is also evidence that DNA repair plays a role in determining response to chemotherapy (Nio et al., 1998, Nio et al., 2001) and that genotype for various DNA repair genes may thereby affect survival of pancreatic cancer patients (Li et al., 2006b, Li et al., 2006a).

Various prospective cohort studies have estimated a 2-3 fold increased risk of the development of pancreatic adenocarcinoma in smokers and that the risk increases with the number of cigarettes smoked and the duration of smoking (Engeland et al., 1996, Fuchs et al., 1996, Harnack et al., 1997, Nilsen and Vatten, 2000). Its relationship with smoking (nearly 25 - 30% of pancreatic cancers are thought to be smoking related (Fuchs et al., 1996), which is potentially the most important avoidable cancer risk in humans, provides us with a unique opportunity to reduce incidence. This aetiological relationship also provides an opportunity to examine the role of tobacco derived carcinogens and gene-environment interaction in the development of pancreatic cancer. Better understanding of pancreatic carcinogenesis will hopefully contribute to its earlier diagnosis and improved outcome.

Increasing work is now being carried out into genetic susceptibility factors responsible for tobacco-related malignancy and specifically into genetic polymorphisms and the risk of pancreatic cancer. The results up to now have been inconsistent. This is probably because most studies have been small and limited to single centres. Also in most cases the gene variants studied have been single polymorphisms of one gene from one pathway out of the multiple pathways involved in cancer development. It is difficult to detect the difference a SNP makes to the phenotype i.e. development of cancer, which involves multiple mechanisms and pathways regulating absorption, metabolic activation and excretion of carcinogens, DNA repair, cell-cycle control and regulation of the local environment etc (Wu et al., 2004). Studying multiple SNP's from a single pathway, therefore, may be more beneficial in being able to elucidate the role that the particular pathway plays in the development of that particular cancer. Obviously the pathway of study needs to be selected carefully, taking into account the individual environmental, genetic and epigenetic events relevant to the organ. In the case of pancreatic cancer, DNA repair mechanisms appear to be good candidates for further study.

Pancreatic ductal adenocarcinoma is a disease with an overall poor prognosis mainly due to its late presentation. The best approach towards this malignancy is prevention. It is possible that a significant difference to outcome from pancreatic cancer will be made through earlier diagnosis. Delineating the role of gene-environment inter actions in pancreatic carcinogenesis will be the key to stratifying risk for individuals and thereby improving survival not only by earlier diagnosis but also by modifying treatment based on individual genotype.

Chapter 2: Research Study and Methods

Section 2.1 Aims

The aims of this study were to

1) Ascertain risk conferred by tobacco smoking and a family history of malignancy for development of pancreatic cancer
2) Explore the relationship and interaction between tobacco smoking and a family history of cancer between pancreatic cancer cases and controls
3) Quantify risk for pancreatic cancer by genotype for polymorphisms in genes relevant to DNA repair, namely hOGG1 Ser326Cys, APE/APEX1 Asp148Glu, XRCC1 Arg194Try, XRCC1 Arg280His and XRCC1 Arg399Gln.

Section 2.2 METHODS

This is a prospective case-control study in patients with ductal adenocarcinoma of the pancreas. Cases were index patients with pancreatic adenocarcinoma presenting to the Hepato-Pancreato-Biliary (HPB) Unit at the Freeman Hospital. Our Unit is the tertiary referral centre for HPB illnesses in the North of England and we cater to a population of 3.5 million. The large numbers of patients with pancreatic cancer being assessed and treated here made this study design possible.

Most diagnoses were confirmed either histologically or cytologically. In some patients we have accepted radiological features in combination with clinical progression and biochemical evidence of malignancy.

Controls were identified from patients presenting to the Freeman Hospital with non-malignant illnesses of the pancreas and liver and with other medical illnesses. We also approached the spouse of the index case patient for participation in our study as controls. These patients recruited, as controls were those scheduled for elective non-urgent surgery e.g. hernia operations, varicose vein surgery, day-case local anaesthetic procedures like removal of sebaceous cysts etc. In addition we identified patients attending the anti-coagulation clinic at the Freeman Hospital to discuss possible participation in our study. These were carefully screened to ensure that they did not have malignancies and or chronic long-term illnesses. Most of these patients were individuals with cardiac rhythm abnormalities on anti-coagulation. We have deliberately excluded patients with peripheral vascular disorders and also patients with suspected urological malignancies. This is due to the high incidence of tobacco smoking in patients with peripheral vascular disease and various recent studies implicating deficient DNA repair in bladder and prostate malignancies and as such their inclusion might have led to a selection bias amongst controls. We also excluded individuals as controls if any

of their first degree relatives were currently being treated at our Unit for Liver/Pancreas/Gall bladder related problems to address the issue of relatedness between cases and controls. Controls were recruited from hospital patients due to limitations on finances, time and logistics. Ideally an age and sex matched population based healthy control group should have been recruited but this was not possible.

2.2.1 Recruitment of cases

The criteria for recruitment of cases was

1) Cytological or histological confirmation of ductal adenocarcinoma of pancreas or

2) Clinical findings and progressive radiological disease with biochemical (tumour marker) evidence of ductal adenocarcinoma of pancreas.

Suitable patients were approached and the research project was discussed with them. A patient information sheet was provided which served to reinforce the oral discussion. Contact numbers were provided to the patient in case he/she needed further information/wished to retract the consent for the study. After securing informed written consent a detailed tobacco-smoking questionnaire was administered and further details on alcohol consumption, occupation and family history of any neoplasms were recorded.

Ethical approval for this project was secured in June 2005 from Gateshead LREC and recruitment commenced immediately.

2.2.2 Questionnaire on Tobacco smoking and Family history of cancer

The World Health Organization Monitoring of Cardio-vascular risks (MONICA) questionnaire was used to record detailed tobacco exposure. The MONICA (Multinational **MONI**toring of trends and determinants in **CA**rdiovascular disease) This project was established in the early 1980s in many centres around the world and administered by the WHO to monitor trends in cardiovascular diseases, and to relate these to risk factor changes in the population over a ten-year period. We have used their validated smoking questionnaire (MONICA smoking questionnaire 1992 Appendix 5) to collect data on tobacco smoking. This enabled us to calculate cumulative tobacco exposure in individuals and to arrive at total pack-years of exposure. Individuals were considered smokers (current and ex) if they had smoked at least 100 cigarettes in their lifetime and non-smokers if they had not smoked this amount. They were considered ex-smokers if they had stopped smoking for a period of one year prior to recruitment.

A detailed family history relating to malignant disease in their first-degree relatives was also obtained directly from the patient. First-degree relatives were defined as biological parents, siblings and offspring. Cases and controls were divided into 2 groups on the basis of a positive family history in first-degree relatives (FDR): FDR+, in whom there was history of malignancy (other than dermatological and primary brain malignancies) in first degree relatives; and FDR-, in whom there was no such history. FDR1 denoted index cases with a single FDR with malignancy; FDR>1 denoted those with more than one FDR with malignancy. However we have not secured information on number of FDR's, their ages at diagnosis of cancer (if any) and their current health status. This has implications, as we are not able to calculate person-years of risk.

Although heavy alcohol consumption is now a known risk factor, (Tramacere et al., 2010) for pancreas cancer data on alcohol consumption was not collected in a detailed

and comprehensive manner when our cases and controls were recruited (2005-06). However an attempt was made to collect information on alcohol consumption but due to various factors including lack of time, logistics and importantly the absence of a structured questionnaire, which measures long-term alcohol consumption, this was not possible. Also in the patients in whom we attempted this, we found that the individuals found it difficult to recall details regarding alcohol consumption from many years ago and the information obtained was really not of much use for scientific analysis.

We also collected data on the mode of diagnosis of the adenocarcinoma of pancreas.

2.2.3 Collection of peripheral blood

A sample of peripheral blood was collected into 2 separate EDTA tubes (resistant to -80° C), at time of phlebotomy for routine investigations/when intravenous access was obtained for treatment purposes. In a few instances venepuncture specifically for the purpose of obtaining blood for the research was performed.

2.2.4 Storage of samples

After appropriate coding the peripheral blood samples were stored in a secure -80° C freezer until DNA extraction. A database was maintained with records of the details of the patient with the code relating to the sample.

2.2.5 Preparation of DNA from peripheral blood

DNA from peripheral blood was prepared exactly as described earlier (Daly et al., 2006). It involved lysing 5 ml blood with 35 ml cell lysis buffer containing 10 mM Tris-HCl pH 7.4, 320 mM sucrose, 5 mM magnesium chloride and 1% Triton-X100 followed by centrifugation at 3000rpm for 10 minutes. The nuclear pellet was resuspended in nuclear lysis buffer (2 ml), which contained 400 mM Tris-HCl pH7.4,

60 mM EDTA, 150 mM sodium chloride and 1% sodium dodecyl sulphate. 0.5 ml 5 M sodium perchlorate was then added. The sample was rotary mixed at room temperature for 15 min and then incubated at 65 C for 30 minutes. To this 2 ml chloroform was added and further rotary mixing (at room temperature) was carried out. The sample was centrifuged at 3000g for 10 minutes when the DNA containing phase came out uppermost. This phase was transferred to another 15 ml tube and 2 volumes of 100% ethanol was added. The DNA came out of solution on gentle inversion and could be spooled out on to a loop. This was air dried and dissolved in 0.2-0.4 ml of 10 mM Tris-HCl pH 7.4 buffer by incubating at 60° C overnight. The sample was then stored at 4° C. The DNA concentration was determined by use of a Nanodrop spectrophotometer to measure the absorbance at 280 nm.

2.2.6 Genotyping protocols

Following extraction of DNA, a private company KBioSciences was engaged to perform genotyping for all samples by an allelic discrimination technique (http://www.kbioscience.co.uk/lab%20services/SNP%20Genotyping/xml%20data.html. Details of the polymorphism and DNA samples were provided to the company who generated a spreadsheet of results. We however performed PCR-RFLP on selected samples (every fifth) to confirm results from KBioSciences. All genotyping data used for statistical analysis was that obtained from the genotyping analysis performed by KBioSciences.

2.2.6.1 Polymerase Chain Reaction methodology

50ng of genomic DNA was amplified in a total reaction volume of 25 µl, containing 1 X reaction buffer (Promega, UK), 0.25 µM forward primer, 0.25 µM reverse primer (Eurofins DNA, UK), 0.2 mM dNTPs (VH Bio) and 0.5 U Taq polymerase (New

England Biolabs) and 1.5 mM $MgCl_2$. Reactions were performed in 0.2 ml thin walled, flat top sterile tubes (Fisher Scientific). Thermal cycling was conducted in an Applied Biosystems 2720 Thermal Cycler. All PCR conditions were as published earlier by Hu et al (Hu et al., 2001) and by Tuimala et al (Table 2.1 – at end of Section). The efficiency of PCR was checked by running 5 μl of the PCR product with a 2Log ladder on 2% agarose gel (see below). Assays were typically run in batches of 20 with each set including 3 controls of known genotype.

2.2.6.2 Primers

Primers were purchased from Eurofins DNA, UK. The lyophilised primers were re-suspended to 200 μM with sterile water for injection (Fresenius Kabi Limited, Chesire, UK). Working 25 μM stock dilutions were made up and kept at 4°C, whilst frozen stocks were kept at -20°C.

2.2.6.3 Restriction fragment length polymorphism analysis

Restriction enzyme digestion of PCR products

Restriction enzymes were purchased from either Fermentas or NEB. Typically 2 U of enzyme was used to digest 20 μl PCR product in the digestion buffer provided, overnight at the temperature specified by the manufacturer.

Electrophoresis, PAGE and visualisation of DNA

Agarose gel electrophoresis

2% agarose gels were made using DNase and RNase free agarose tablets (Bioline,UK) in 1 X TBE, containing ethidium bromide (0.5 μg/ml). 2 μl of gel loading dye was added to 5 μl of DNA product, and samples were loaded alongside 2 Log Ladder (NEB)

for size determination of the products. Electrophoresis was generally carried out for 15-30 minutes at ~80V.

Polyacrylamide gel electrophoresis (PAGE) and ethidium bromide staining

10% (w/v) polyacrylamide gels were made using 30% (w/v) acrylamide-bis acrylamide 29:1 (Fisher) in 1X TBE buffer, 0.4 mg/ml ammonium persulphate and 0.1% (v/v) TEMED, to a final volume of 50 ml. The gel solution was poured between two 200mmx200mm sealed glass plates and left to set for 30 minutes. 3 μl of gel loading buffer was added to 25 μl DNA digestion products and applied to the wells. Gels were run at 150V for 4-6hrs. Gels were then post-stained in 1XTBE and ethidium bromide (0.5 μg/ml) for 30mins.

Gel visualization

Gels were visualised and photographed on a BioRad gel documentation system and photographed using Fluor S-Multi imager Quantity One software.

hOGG1

KBiosciences performed genotyping for all samples by an allelic discrimination technique for a tagged SNP rs2072668**.** In some selected samples genotyping of this polymorphism was performed by PCR-RFLP as described in Section 2.2.6 to confirm the KBiosciences data was correct.

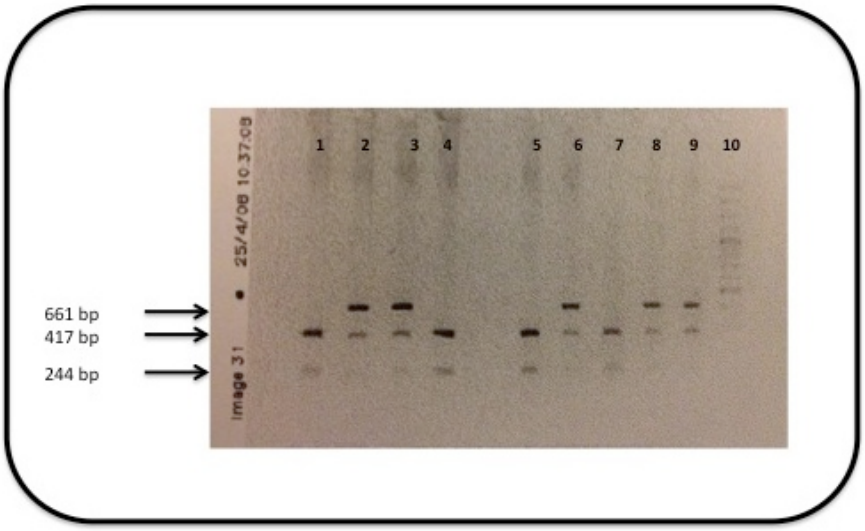

Figure 2.1: hOGG1 Ser326Cys PCR-RFLP

A typical digestion pattern is depicted where FNU4H1 digested products of hOGG1resoved at 691, 691/417/244 and 417/244; lanes 1,4,5,7 are homozygous mutants whilst lanes 2,3,6,8,9 are heterozygotes.

XRCC Arg194Try

KBiosciences performed genotyping for all samples by an allelic discrimination technique. In some selected samples genotyping of this polymorphism was performed by PCR-RFLP as described in Section 2.2.6 to confirm the KBiosciences data was correct.

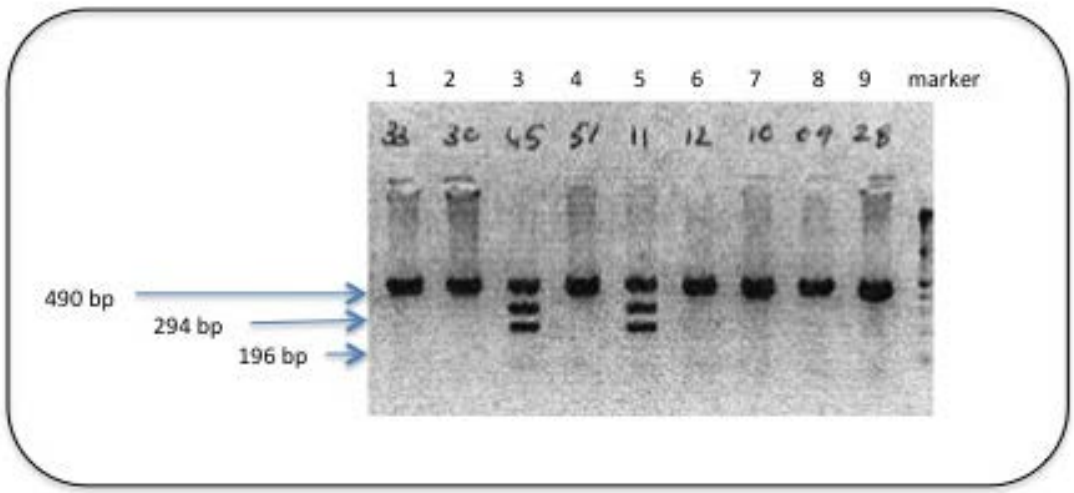

Figure 2.2: XRCC1 Arg194Try PCR-RFLP

A typical digestion pattern is shown where Pvu2 digested products of XRCC1 codon 194 Arg/Arg, Arg/Trp and Trp/Trp resolved at 490, 490/294/196 and 294/196 bp on 2% agarose gel. Therefore lanes 3 and 5 are heterozygotes while the rest are homozygous wild-type genotype. Lane 10 is a 100 bp marker.

XRCC Arg399Gln

KBiosciences performed genotyping for all samples by an allelic discrimination technique. In some selected samples genotyping of this polymorphism was performed by PCR-RFLP as described in Section 2.2.6 to confirm the KBiosciences data was correct.

A typical digestion pattern is shown in Figure 2.3

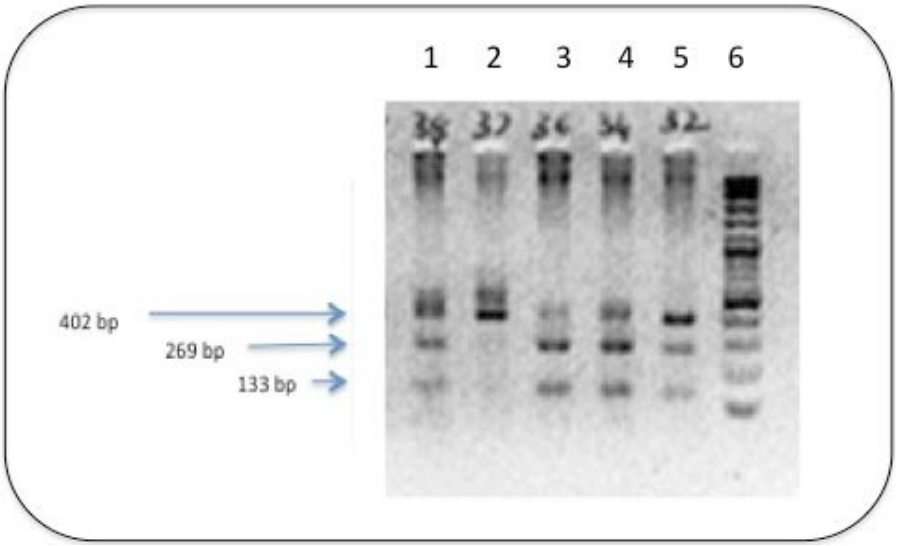

Figure 2.3 XRCC1 Arg399Gln PCR-RFLP

Hpa 1 (isoschizomer of Msp1) resolved products of XRCC1 codon 399 Arg/Arg, Arg/Gln and Gln/Gln had band sizes of 269/133, 402/269/133 and 402 bp on 2% agarose gel. The wild type gene is digested by Hpa1, therefore sample 38, 36 and 34 in lanes 1, 3 and 4 are homozygous wild-type genotype, while sample 37 in lanes 2 is a homozygous mutant and sample 32 in lane 32 is a heterozygote. Lane 11 is a 100bp marker.

XRCC 280

KBiosciences performed genotyping for all samples by an allelic discrimination technique. A few selected samples of XRCC1 Arg280His were genotyped by PCR-RFLP as described in Section 2.2.6.

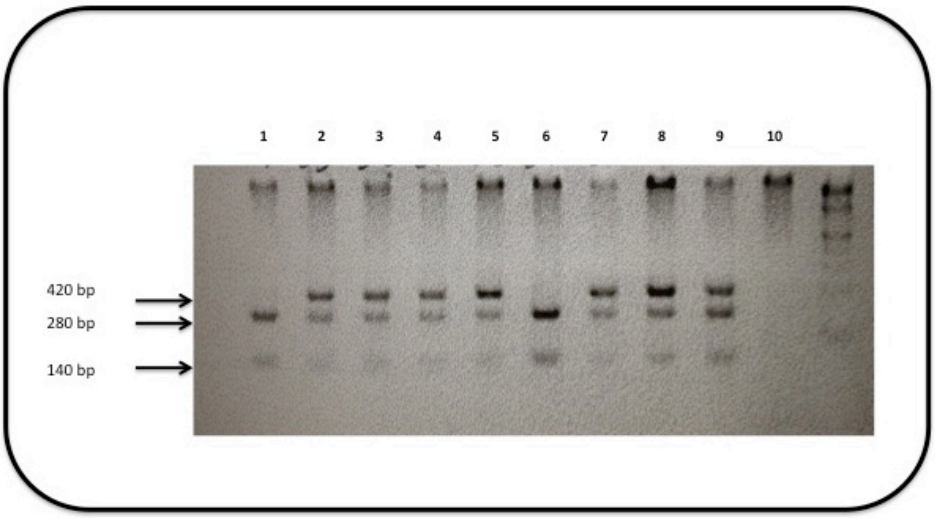

Figure 2.4 XRCC1 Arg280His PCR-RFLP

A sample digestion pattern is depicted. The digestion products resolved at 420, 420/280/140, 280/140 bp sizes, lanes 1 and 6 are homozygous mutants whilst lanes 2, 3, 4, 5, 7, 8 and 9 are heterozygotes.

2.2.7 Power calculation

Using an online statistical power calculator (http://statpages.org/proppowr.html), a power calculation was performed for each individual polymorphism based on the number of samples available before the genotyping studies were initiated.

hOGG1 Ser326Cys :The use of 67 cases and 67 controls provided a statistical power of 80% to detect a change in the proportion of the frequency of the minor allele from 29% to 58 % [Odds ratio (95% CI) 3.38 (1.8803 to 6.0795)] at a significance level of p=0.01.

XRCC1 Arg194Try The use of 142 cases and 142controls provided a statistical power of 80% to detect a change in the proportion of the frequency of the minor allele from 7% to 21 % [Odds ratio (95% CI) 3.53 (1.42 to 8.74)] at a significance level of p=0.01.

XRCC1 Arg280His The use of 130 cases and 130 controls provided a statistical power of 80% to detect a change in the proportion of the frequency of the minor allele from 15% to 33 % [Odds ratio (95% CI) 2.42 (1.22 to 4.81)] at a significance level of p=0.01.

XRCC1 Arg399Gln The use of 143 cases and 143 controls provided a statistical power of 80% to detect a change in the proportion of the frequency of the minor allele from 35% to 55 % [Odds ratio (95% CI) 2.26 (1.28 to 4.01)] at a significance level of p=0.01.

APE1 Asp148Glu The use of 139 cases and 139 controls provided a statistical power of 80% to detect a change in the proportion of the frequency of the minor allele from 49% to 70 % [Odds ratio (95% CI) 2.42 (1.35 to 4.33)] at a significance level of p=0.01.

Table 2.1: Genotyping details - PCR and digestion conditions for individual genes

Gene	PCR conditions		Digestion conditions			
	Primers	Temperatures	Digestion enzyme	Visualization of DNA	Band sizes	Genotype
hOGG1 Ser326Cys	F: 5'-GGAAGGTGCTTGGGGAAT-3' R: 5 –ACTGTCACTAGTCTCACCAG-3'	35 cycles of 94° C for 1 minute 63° C for 1 minute 72° C for 7 minutes 72° C for 7 minutes	Fnu4H1	Electrophoresis on 2% agarose gel	417 bp 417/244 661	Homozygous wild type Heterozygote Homozygous mutant
XRCC1Arg194Try	F 5'GCCCCGTCCCAGGTA3' R 5'AGCCCCAAGACCCCTTTCACT3'	95 C for 2 minutes, Followed by 40 cycles of 94 C for 15 seconds, 57 C for 45 seconds 72 C for 45 seconds 1 cycle at 72 C for 5 minutes	Pvu2	Electrophoresis on 2% agarose gel	490, 490/294/196 294/196	Arg/Arg, Arg/Trp Trp/Trp
XRCC1 Arg280His	F 5'TGGGGCCTGGATTGCTGGGTCTG3' R 5'CAGCACCACTACCACACCCTGAAGG3'	95 C for 2 minutes, Followed by 40 cycles of 94 C for 15 seconds, 57 C for 45 seconds 72 C for 45 seconds 1 cycle at 72 C for 5 minutes	Rsa1	Electrophoresis on 3% agarose gel	140 420/280/140 280	Arg/Arg Arg/His His/His
XRCC1 Arg399Gln	F 5'TCTCCCTTGGTCTCCAACCT3' R 5'AGTAGTCTGCTGGCTCTGG3'	95 C for 2 minutes, Followed by 40 cycles of 94 C for 15 seconds, 57 C for 45 seconds 72 C for 45 seconds 1 cycle at 72 C for 5 minutes	Msp1	Electrophoresis on 2% agarose gel	269/133, 402/269/133 402	Arg/Arg, Arg/Gln Gln/Gln
APE1 Asp148Glu	F 5'CTGTTTCATTTCTATAGGCTA3' R 5'AGGAACTTGCGAAAGGCTTC3'	95 C for 2 minutes, Followed by 40 cycles of 94 C for 15 seconds, 57 C for 45 seconds 72 C for 45 seconds 1 cycle at 72 C for 5 minutes	Bfa1	Electrophoresis on 10% polyacrylamide gel	164, 164/144/20 and 144/20	Asp/Asp, Asp/Glu Glu/Glu

2.2.8 Statistical analysis

Continuous variables (checked for normality or otherwise by Shapiro-Wilk test) were compared by the student t-test and ANOVA for parametric variables and the Mann-Whitney U test and Kruskal-Wallis tests for non-parametric variables. Associations were tested using the by Fisher's test (2-tailed p) and Pearson's chi-square test. Goodman and Kruskal Tau/Somers gamma was used when ordinal data were used in cross-tabulation. Directional measures were employed as necessary. To confirm the data was in Hardy-Weinberg equilibrium, an online calculator available at http://www.oege.org/software/hwe-mr-calc.html (Rodriguez et al., 2009) was used.

Odds ratios with 95% confidence interval were calculated to quantify relative risk for individual enzyme polymorphisms and statistical significance was assessed. In addition, conditional logistic regression was used to adjust for effects of age (used as a categorical variable), gender, family history and smoking status between cases and controls. Hosmer Lemeshow test was used to check the model for adequacy of fit during logistic regression. We did not use any censoring in our analysis. Regarding age of individuals (FDRs), we did not have age of onset of cancer in them and therefore were not able to calculate person-years of risk for FDR's.

SPSS for Windows (Rel. 16.0.1. 2008. Chicago: SPSS Inc) was the software platform used for computing these tests. Professional advice was sought on the use of statistical tests and method of analyses (specifically including aspects of data coding, cross-tabulation, significance testing, regression analysis) on the SPSS platform from Laerd Statistics (https://statistics.laerd.com/).

CHAPTER 3: RESULTS - INFLUENCE OF A CUMULATIVE TOBACCO EXPOSURE AND FAMILY HISTORY OF CANCER IN INDEX CASES OF PANCREATIC CANCER

3.1 Introduction

Over a period of 14 months (June 2005 to September 2006), both cases and controls were recruited into this study and here we present the demographics and explore the data obtained.

A total of 181 patients were referred to the Unit with a suspected diagnosis of pancreas cancer and 178 agreed to take part in the study. Out of these, 13 were found to have diagnoses other than ductal adenocarcinoma of the pancreas. One patient with a diagnosis of Li-Fraumeni syndrome was excluded from the data analysis, since this condition predisposes to pancreatic cancer. Therefore there were 164 cases in the study. The mode of diagnosis of pancreatic malignancy was: cytological and or histological evidence of pancreatic ductal adenocarcinoma in 122 patients (74%) and a combination of radiological and/ clinical progression with biochemical (serially rising CA19-9) evidence in 42 (26%) patients (Fig 3.1).

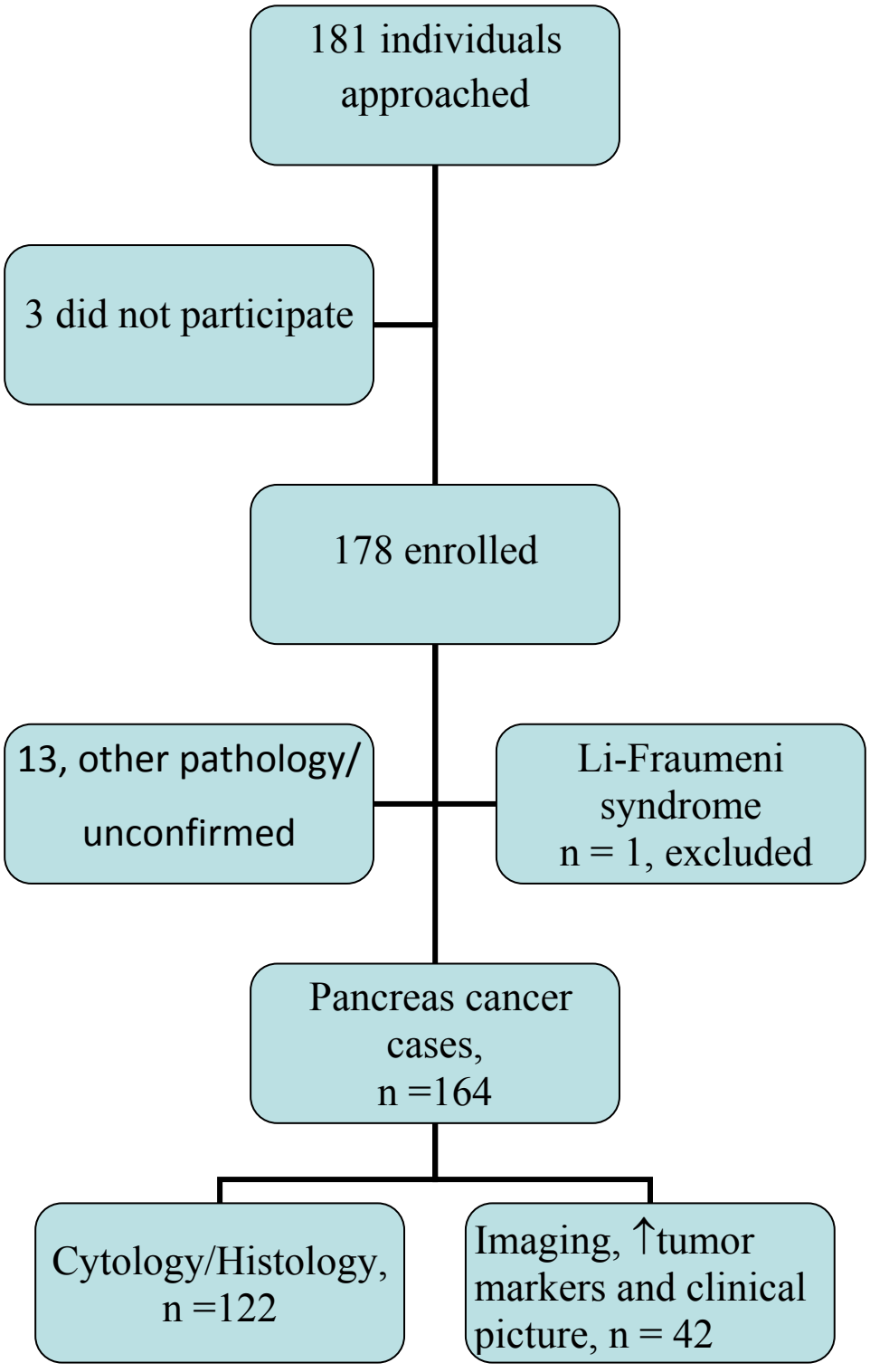

Fig 3.1: Chart depicting enrolment and mode of diagnosis of cases

We recruited 126 controls from the Freeman Hospital. They included spouse controls, patients from the in-patient wards and outpatient clinics. Spouse controls were n =34. The majority of these were individuals attending the anti-coagulation clinic (indications included cardiac arrhythmia, prosthetic cardiac valves; n = 75), patients attending for elective hernia repair surgery (n=5), cholecystectomy (n=8) and endoscopic treatment of bile duct stones and/or benign biliary strictures (n=4). Later in the study period we included 50 controls from an erstwhile molecular epidemiological study, from whom we had consent to do so (*historical controls*). These individuals had provided blood samples from which DNA had been isolated and stored. They had also provided tobacco smoking status information, although detailed data on intensity of the exposure was not available. There was also no information on age, gender and family history of malignancies. Therefore these controls were included only for genotyping analysis and they had to be excluded for other analyses (this is detailed in subsequent sections)

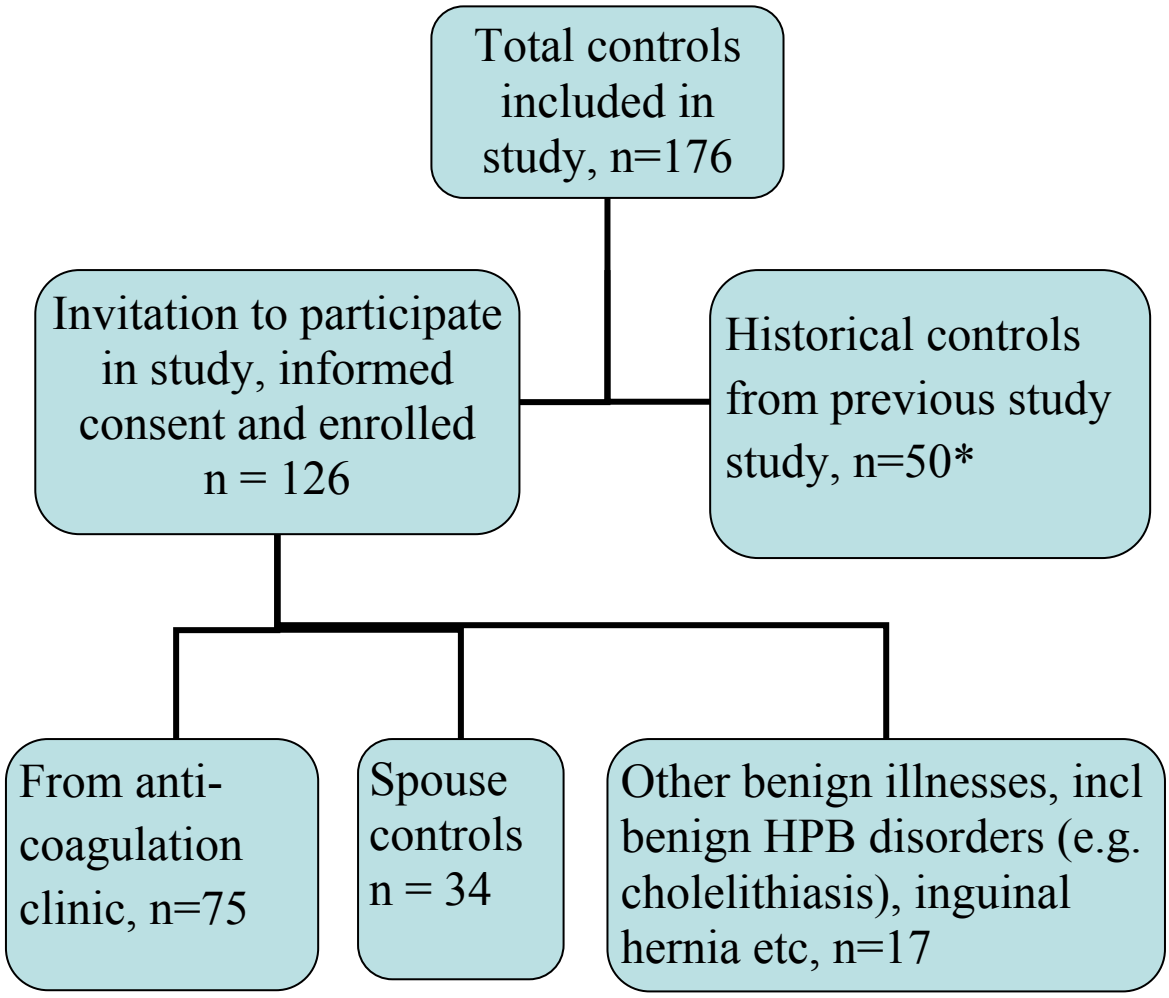

Fig 3.2: Chart depicting enrolment and inclusion of controls into study

* Historical/MW controls

3.2 Cases and controls

3.2.1 Gender

Table 3.1 depicts the frequency of each gender class amongst cases and controls

Table 3.1: Gender grouping amongst cases/controls

		Frequency	Percent
Control	Female	70	39.8
	Male	56	31.8
	Not av*	50*	28.4
	Total	176	100.0
Case	Female	73	44.5
	Male	91	55.5
	Total	164	100.0

*These are historical controls included later in the study on which gender information was not available. In analyses, which have included gender as a variable, we have excluded these controls, and Fisher's test, p=0.075. Therefore there is no association between case/control status and gender.

3.2.2 Age:

We did not have age information on the historical controls (n=50) included in the latter period of the study; we have therefore excluded these individuals from analyses where age was a variable. The data were distributed in a non-normal fashion (Shapiro-Wilk test p=0.001). The mean (95% CI) age of cases (n=164) was 65.36 (63.69 to 67.03) years, whilst that for the controls (n=126) was 61.93 (59.58 to 64.28) years (Mann-Whitney test p=0.040) The median ages were 64 (34 to 87) and 66 (36 to 88) years respectively. Again this finding is probably secondary to the design of the study and not a result of the same.

3.2.3 Smoking status:

There were some individuals from whom a complete tobacco smoking history could not be obtained; from the *historical controls* we could only obtain information on smoking status (present, past or non-smoker). These subjects did not have complete tobacco exposure history and therefore although they were included in analyses in which smoking status was a variable, we excluded these controls when smoking intensity (or other tobacco exposure details) was a variable. In some the MONICA questionnaire was incompletely filled and these are depicted in the penultimate row of Table 3.2.

Table 3-2: Tobacco smoking status

		Control	Case	Total
Smoking status	Present-smoker	67	85	152
	Past-smoker	45	59	104
	Non-smoker	44	10	54
	Incomplete data	20	10	30
	Total	176	164	340

Cross-tabulation suggests a relationship between case/control status and smoking status, Ordinal Chi-square p=0.0001 (very weak relationship; lambda =0.195, case/control status dependent variable). Telescoping smoking status into 2 categories – Ever smokers (group consisting of past and present smokers) vs. Non-smokers and excluding subjects who have incomplete data, we obtain a p=0.0001 (Fishers test) and Odds Ratio (95% confidence interval) [OR (95% CI)] for smoking for development of pancreas cancer = 3.429 (1.612 to 7.291). This suggests that tobacco smoking is a risk factor for pancreas cancer in our study population.

Comparing intensity of smoking in terms of pack-years of tobacco exposure between the 3 smoking statuses (Table 3.3):

Table 3.3: Tobacco exposure (pack-years of smoking) Cases and Controls (excluding missing data)

		N	Mean	Std. Deviation	Mann-Whitney test p
Ever Smokers Pack yrs	Control	112	36.3329	25.37667	0.120
	Case	144	47.1324	38.25516	
Present Smokers Pack years	Control	67	37.8875	25.11526	0.200
	Case	85	50.3047	40.29715	
Past Smokers Pack yrs	Control	45	34.4207	25.89296	0.725
	Case	59	39.4262	35.11719	

Smoking intensity is distributed in a non-normal manner (Shapiro-Wilk; Ever Smokers pack years p= 0.0001, Present Smokers Pack-years p = 0.0001, Past Smokers Pack years p = 0.0002).

All individuals with missing values were excluded for this analysis, and there was no significant difference between tobacco exposure between ever-smokers amongst cases (47.13 pack-years) and controls (36.33 pack-years). This was true for present and past smokers too.

3.2.4 Family history:

Of the 164 cancer patients, 134 with reliable family history were included into this analysis (family history data being unavailable in 21 and incomplete in 9). A full smoking history was available in all these individuals. *Historical controls* were excluded from this part of the analysis, as we did not have family history data on them. Table 3.4 depicts the frequencies of individuals with a positive (FHP), negative (FHN), incomplete (FHIncomp) and unavailable (FHUnavail) family history of cancer in their first-degree relatives.

Table 3.4: Family history of malignancy amongst Cases and Controls

		Family history				
		FHP	FHN	FHIncomp	FHunav	Total
Cases & Controls	Case	78	56	16	14	164
	Control	39	70	15	52*	176
	Total	110	133	31	66	340

**historical controls included*

Excluding missing values (FHIncomp and FHUnav), there is a significant relationship between family history of malignancy and case/control status (Fisher's test p=0.041) and calculating odds ratio for individuals with family history of malignancy we obtain OR (95% CI) of 2.023 (1.205 to 3.396). However this analysis is limited by the non-availability of person-years of risk in the FDR's.

There were 78 cases with FDR+ (first degree relatives with a positive) for history of malignancy with a total of 107 malignancies; FDR's with 1 malignancy (FDR1) n = 51, 17 had 2 relatives, 6 had 3 and one had 4 relatives with cancer. There were 39 FDR+ controls with a total of 59 malignancies; FDR1 n = 39 and FDR1+ = 12. The following Figure 3.3 details the frequency of the individual's cancer types amongst FDR+ cases and controls.

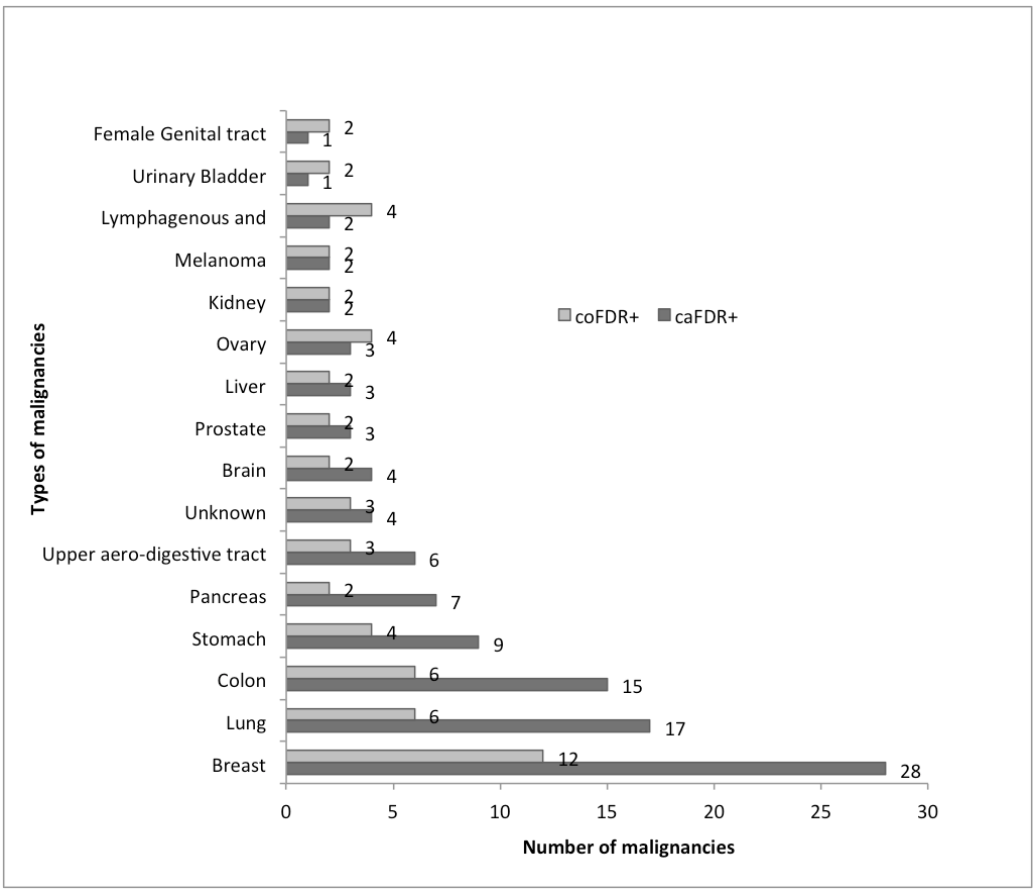

Figure 3.3: Types of malignancies in caFDR+ (n=78) & coFDR+ (n=39)

Tobacco smoking is a risk factor for pancreas cancer and so is family history of malignancy (vide supra). The relationship between these factors was investigated by comparing groups of patients with tobacco exposure but with different genetic backgrounds i.e. family history.

The mean (SD) age at diagnosis for pancreatic cancer cases was 66.1 (10.67) years. There was no difference (p=0.35) in the mean (SD) age between caFDR+ and caFDR- groups (65.93 (8.90) and 62.23(13.65) years respectively). The overall gender ratio was 77:57 (m: f), (45:32 for caFDR+ and 32:24 for caFDR-).

The controls numbered 172, of which 119 were included for this analysis due to constraints of reliability or completeness of family history: coFDR+ = 44, coFDR- = 75,

unavailable = 5, incomplete = 7. Mean age of controls was 62.07 (14.34) years. There was no significant difference between the ages of coFDR+ and coFDR- groups. The overall gender ratio was 60:59 (m: f) (25:19 for coFDR+ and 39:36 for coFDR-).

Table 3.5 summarizes the demographics, smoking behaviour, cumulative tobacco consumption, (overall consumption and stratified by FDR status, of our study population (total 253; cases 134 and controls 119).

3.3 Tobacco exposure and risk of pancreatic cancer

There were 114 pancreatic cancer patients who had experienced significant tobacco exposure at some point in their lives; 70 were current smokers and 44 were ex-smokers who had stopped smoking at a mean (SD) of 19.19 (14.48) years prior to diagnosis of adenocarcinoma of pancreas. The mean (SD) cumulative tobacco exposure in these 97 individuals was 37.62 (22.45) pack-years. There were 20 non-smokers. The mean (SD) cumulative tobacco exposure in all controls that had experienced tobacco exposure (n = 78, current smokers = 47 and ex smokers = 31) was 22.45(29.04) pack-years and this was significantly lower (p=0.002) than that in pancreatic cancer cases. There were 41 non-smokers amongst the control population. There was no significant difference in the number of past smokers between the cases and controls but significant differences were seen in the numbers of Ever- and non-smokers (Table 3-5 and Figure 3.4).

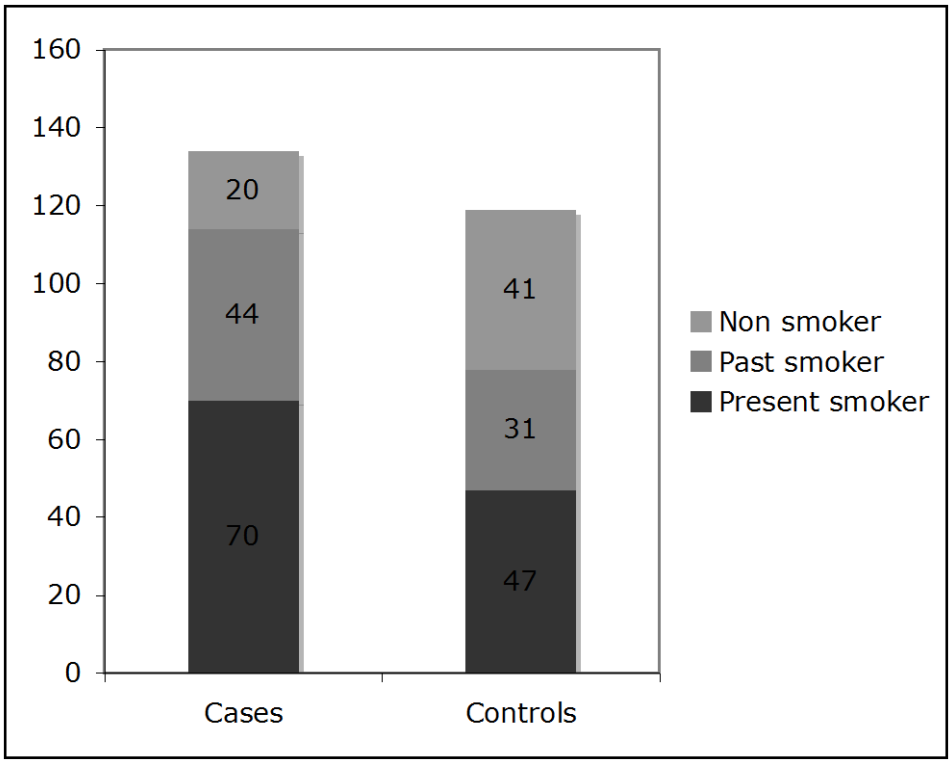

Figure 3.4: Frequency of Tobacco smokers - Cases and Controls

Table 3-5: Detailed tobacco exposure data - cases and controls stratified by Family history

	Cases	Controls	p value
Total	164	176	
Included into analysis	134	119	
Male	77	60	0.312 (Fisher's test)
Female	57	59	
Mean Age	66.1 (10.67)	62.07(14.34)	0.421 (t-test)
Current smokers	70	47	0.881 (Fisher's Test)
Past smokers	44	31	
Ever-smoker (Present + Past)	114	78	0.0004
Non smokers	20	41	
FDR+	78	39	0.0008 (Fisher's test))
FDR-	56	70	
FDR+ Smoking PyYrs	32.79(24.77)*	23.51(12.98)**	0.129 (Mann-Whitney)
FDR- Smoking PackYrs	45.69(24.56)*	19.33(16.11)**	0.001 (Mann-Whitney)
Overall Smoking PackYrs	37.62(22.45)	22.45 (19.04)	0.002 (Mann-Whitney)
	*p = 0.023 (Mann-Whitney)	**p = 0.193 (Mann-Whitney	

No allowance for alcohol exposure is included, as the data on this was not collected (as discussed in 2.2.2 (pages 86/87).

The odds ratio for an ever smoker (current and ex) for the development of pancreatic cancer is nearly 3 times that of a non-smoker [OR 3.01 (95% CI 1.73 to 5.24)]. There was no significant difference in the mean age between the cases and controls; however there was a definite early onset of adenocarcinoma of pancreas in current smokers. A consistent early occurrence of adenocarcinoma of pancreas by about 6 - 7 years was seen amongst current smokers, which is independent of family history of cancer (Table 3.7). However this finding could be a result of the design of the study. FDR status did not affect the age of onset of pancreatic cancer. Again this analysis is hampered by the non-availability of age of onset of the malignancy in the FDR's.

Table 3.6: Age of Onset of adenocarcinoma of pancreas [Mean (SD) years]

Pancreas cancer patients grouped based on family history of malignancy status	Smoking status					
	Current smoker	Current and Ex smoker	Ex-Smoker	Non-Smoker	Ex and non-smoker	ANOVA p
Combined caFDR+ and caFDR- (n=134)	**60.12 (8.18)**		**67.59 (10.10)**	**66.36 (12.17)**		**0.005**
		64.51(10.01)		**66.36 (12.17)**		**0.40**
	60.12 (8.18)				**67.08 (10.95)**	**0.001**

3.4 Family history of cancer in caFDRs

A history of malignancy in FDR was present in 78 (m: f = 44:34) and absent in 56 (m: f=32:24) cases. Amongst controls the coFDR+ numbered 39 and coFDR- was 70. The risk of development of adenocarcinoma of pancreas for caFDR+ individuals was nearly twice that of caFDR- individuals [OR 1.98 (95% CI: 1.15-3.38)]. However we do not have data on the total number of first-degree relatives, the ages at which they developed these malignancies and current health status of these individuals. Therefore we are not able to calculate life-time risk.

Of the 78 cases caFDR+, 51 had a single relative with cancer, 17 had 2 relatives, 6 had 3 and one had 4 relatives with cancer. In total there were 107 malignancies. The different malignancies in the caFDR+ group are depicted in Figure 3-3.

3.5 Interaction between tobacco smoking and family history of cancer in caFDRs

The overall tobacco exposure was greater amongst cases (Mann-Whitney test p=0.002,) as seen in Table 3-5. More importantly amongst cases, there was a significantly decreased cumulative tobacco exposure in the caFDR+ group (Mann-Whitney test p=0.023) as compared to the caFDR- group: the overall cumulative pack-years of smoking was 32.79 (24.77) in the caFDR+ versus 45.69 (24.56) in the caFDR- group. Mean (SD) cumulative tobacco exposure in coFDR+ was 23.51(12.98) and that in coFDR- was 19.33 (16.11). This was not statistically different (Mann-Whitney test p=0.193) [Table 3.5]. The risk for adenocarcinoma of the pancreas was higher in smokers in both FDR+ [OR 2.85(95% CI 1.24 to 6.65)] and FDR- [OR 3.18 (95% CI 1.48 to 6.82)] groups, but the amount of tobacco exposure was lower in the caFDR+.

Next we divided the cases with a family history of cancer in their FDR into 2 groups – caFDR 1 (n=36): one FDR with cancer and caFDR>1 (n=24): cases with more than 1 FDR with cancer. We did not find a significant difference in the mean (SD) cumulative pack years of tobacco smoking in between these groups [FDR1: 33.70 (29.24), FDR2: 25.07 (16.68); Mann-Whitney test p = 0.269].

3.6 Summary of results:

1. The risk for an ever smoker (current and ex) for the development of pancreatic cancer is nearly 3 times that of a non-smoker [OR 3.01 (95% CI 1.73 to 5.24)].

2. Amongst pancreatic cancer cases, there was a significantly decreased cumulative tobacco exposure in the caFDR+ group (p=0.023) as compared to the caFDR- group: the overall cumulative pack-years of smoking was 32.79 (24.77) in the caFDR+ versus 45.69 (24.56) in the caFDR- group.

3. Therefore, although we have attempted to examine family history of malignancy in the context of tobacco smoking towards risk for pancreatic cancer these results are severely limited by the limited data available on the total number of FDR's in a family and their current health status. The findings from this study need to be replicated in a further robust data collection exercise where a comprehensive family history will be collected, including number of FDRs, age at diagnosis of malignancy in them and current health status of the individuals which will allow calculation of person-years of risk and Standardised incidence ratios.

CHAPTER 4: GENOTYPING RESULTS

Section 4.1: rs2072668 genotype (hOGG1)

The relevance of OGG1 genotype to pancreatic cancer was assessed by genotyping for rs2072668, which is in linkage disequilibrium with HOGG1 Ser326Cys (rs1052133). rs2072668 is an intronic hOGG1gene polymorphism on chromosome 3 at contig position 9738140 (rs1052133 is at contig position 9738773). KBiosciences performed genotyping for this SNP by allelic discrimation techniques.

Genotypes for rs2072668 in the genotyped population (n=312) - cases (n=153) were in HW equilibrium (X^2=1.95, p=0.162) and controls (n=159) were in HW equilibrium too (X^2 = 0.36, p=0.548). The overall frequencies of wild-type, heterozygote and homozygous mutant genotypes are depicted in Table 4.1. The overall allele frequencies were major allele 497 (79.65%) and minor allele 127 (20.35%), amongst cases they were major allele 247 (80.72%) and minor allele 59 (19.2%), amongst controls they were 250(78.2%) and 68 (21.38%) respectively.

Table 4.1: rs2072668 genotype frequency

		Case	Control	Total
rs2072668 genotype	Missing	11	17	28
	Homozygous Wild-type	97	97	194
	Heterozygous	53	56	109
	Homozygous Mutant	3	6	9
	Total	164	176	340

4.1.1 Overall association between genotype and case/control status

To overcome small numbers in the homozygous mutant category, heterozygotes and homozygous mutants were combined into a single group (Table 4.2).

Table 4.2: rs2072668 Homozygous wild-type vs. Carriers of variant allele

rs2072668	Case Control		
	Case	Control	Total
Carriers of variant allele	56	62	118
Homozygous Wild-type	97	97	194
Total	153	159	312

No association (Fisher's test p=0.726) was seen. The odds ratio for pancreatic cancer was 0.848 (95% CI 0.552 to 1.304), which was not significant.

4.1.2 Assessment of Gender on rs2072668 risk for pancreatic cancer

Performing sub-group analysis based on gender, there was no association between genotype and case/control status (Fisher's test; Female p= 1.000, Male p = 0.627) (Table 4.3). Calculating risk for pancreatic cancer; over all odds ratio for female carriers of variant allele was 0.975 (95% CI 0.502 to 1.894) and for males, was 0.833 (95 % CI 0.41 to1.574). These risk estimates are not significant.

Table 4.3: Comparison of genotype frequencies for rs2072668 in male and female cases and controls

		rs2072668		
		Carriers of variant allele	Homozygous wild - type	Total
Female	Case	25	43	68
	Control	31	52	83
	Total	56	95	151
Male	Case	31	54	85
	Control	31	45	76
	Total	62	99	161

4.1.3 Assessment of Age on rs2072668 risk for pancreatic cancer:

The mean (SD) age at diagnosis for pancreatic cancer cases was 66.1 (10.67) years and that of the controls was 62.07 (14.34) years. We sought to divide our study group into 2 categories to ascertain the impact of age. We chose 65 years as a cut-off as it fell in between the means of the cases and controls and was a round figure. We therefore divided individuals into 2 age groups – up to 65 years and above 65 years.

As summarized in Table 4.4; there were no significant differences in risk for the two age groups (Fisher's test; less than 65 years p=0.862; more than 65 years p=0.743). Odds ratio for pancreatic cancer for the younger age group was 0.914 (95% CI 0.463 to 1.805) and that for the above 65 years was 0.854 (95% CI 0.449 to 1.626).

Table 4.4: rs2072668 genotypes in cases and controls; effect of age

		rs2072668		Total
		Carriers of variant allele	Homozygous Wild-type	
≤ 65 years	Case	32	35	67
	Control	33	33	66
	Total	65	68	133
> 65 years	Case	24	62	86
	Control	29	64	93
	Total	53	126	179

4.1.4 Smoking status:

The relationship between tobacco smoking behaviour and case-control status was assessed. Some individuals (n=28) did not have full smoking history and were excluded from this analysis. The results obtained are shown in Table 4.5.

Table 4.5: rs2072668 genotypes and susceptibility to pancreas cancer - effect of smoking status

		rs2072668		Total
		Carriers of variant allele	Homozygous Wild-type	
Present-smoker	Case	28	51	79
	Control	18	44	62
	Total	46	95	141
Past-smoker	Case	20	36	56
	Control	16	21	37
	Total	36	57	93
Non-smoker	Case	3	7	10
	Control	23	17	40
	Total	26	24	50

Genotype distributions did not differ significantly between individual smoking groups (Fisher's test p=0.417, 0.518 and 0.164 present, past and non-smokers respectively). Odds ratio for pancreatic cancer for individual smoking statuses were 1.342 (95% CI 0.656 to 2.747), 0.729 (95% CI 0.312 to 1.705) and 0.317 (95% CI 0.071 to 1.407).

4.1.5 Smoking intensity

Intensity of tobacco smoking was assessed by dividing smokers into 2 groups, ≤40 pack-years history (light smokers) and above 40 pack-years (heavy smokers) of cumulative tobacco exposure (Table 4.6).

Table 4.6: rs2072668 genotypes in cases and controls - effect of smoking intensity

		rs2072668		
		Carriers of variant allele	Homozygous Wild-type	Total
Light	Case	13	37	50
	Control	12	14	26
	Total	25	51	76
Heavy	Case	38	57	95
	Control	45	68	113
	Total	83	125	208

No significant associations were seen for either group (Fisher's test, light smokers p = 0.121, heavy smokers p=1.000). Odds ratios were 0.410 (95% CI 0.151 to 1.111) for light smokers and 1.007 (95% CI 0.577 to 1.759) for heavy smokers.

4.1.6 Family history of malignancy

The effect of the presence (FHP) or absence (FHN) of a family history of malignancy on case-control status was explored (Table 4.7).

Table 4.7: rs2072668 genotypes in cases and controls - effect of family history of malignancy

		rs2072668		
		Carriers of variant allele	Homozygous Wild-type	Total
FHP	Case	24	41	65
	Control	17	17	34
	Total	41	58	99
FHN	Case	21	38	59
	Control	24	37	61
	Total	45	75	120

Some individuals n=93 did not have a full family history and they were excluded from this analysis. Amongst FHP (Fisher's test, p=0.282) and FHN (Fisher's test, p=0.709) no significant associations were seen for genotype and membership of cases. Odds ratio (95% CI) values were 0.585 (0.253 to 1.356) and 0.852 (0.406 to 1.786) respectively.

4.1.7 Logistic regression

Logistic regression was performed excluding incomplete and missing values (amongst genotype, age, gender, family history and smoking status) to ascertain the impact of these variables on the likelihood of developing pancreatic ductal adenocarcinoma. The total numbers of cases included was 207 (Table 4-8). The Hosmer Lemeshow test gave a X^2= 3.701 (p=0.883) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 39.457 p=0.0009. The model explained 23.5% (Nagelkerke R^2 = 0.235) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 70.5% of cases. Sensitivity was 87.1%, specificity was 45.8%, positive predictive value was 70.58% and negative predictive value was 70.37%. Of the 5 predictor values only age and smoking status were significant – Table 4.8. Increasing age was associated with risk of pancreatic cancer, both present and past smokers were at increased risk but no specific risk was associated with the variant allele for rs2072668. Age (continuous variable) and its logit transformation are not related significantly (p=0.120), therefore they are linearly related confirming that this logistic regression is valid.

Table 4.8: Logistic regression analysis on hOGG1 genotype and relationship to pancreas cancer susceptibility

	B	Sig.	Odds ratio	95% C.I. for odds ratio	
				Lower	Upper
Female Gender	-0.524	0.100	0.592	0.317	1.105
Age	0.037	.008	1.038	1.010	1.067
FHP	0.381	.232	1.464	.783	2.736
Present smoker	2.059	.000	7.840	3.196	19.231
Past smoker	1.570	.001	4.807	1.962	11.774
rs2072668 Carriers of variant allele	-.020	.952	.980	.511	1.878
Constant	-.568	.233	.567		

Section 4.2: XRCC1 (Arg399Gln) rs25487

4.2.1 Overall association between genotype and case-control status

The frequencies of the individual genotypes are depicted in Table 4.9. Both cases and controls were in Hardy-Weinberg equilibrium, cases (X^2 =0.05, p=0.823), controls ($X2$ = 0.68, p=0.409) and the overall frequencies for the major allele was 406 (65.48%) and minor allele 214 (34.52%). The allele frequencies amongst cases were major allele 196 (64.9%) and minor allele 106 (35.1%). Amongst controls this was 210 (66.04%) and 108 (33.9%) respectively.

Table 4.9: XRCC1Arg399Gln genotype frequency

Case Control		Frequency	Percent
Case	Missing	13	7.9
	Homozygous Mutant	18	11.0
	Heterozygous	70	42.7
	Homozygous wild-type	63	38.4
	Total	164	100.0
Control	Missing	17	9.7
	Homozygous Mutant	16	9.1
	Heterozygous	76	43.2
	Homozygous wild-type	67	38.1
	Total	176	100.0

Excluding missing values, the frequency of the variant allele amongst controls; 108/318 =33.9%, which is almost identical to the previously reported 34.7% (95% CI: 33.8 to 35.6) (Hu et al., 2005).

For further analysis, homozygous mutants and heterozygous carriers were combined into a single carrier group. Table 4.10 summarizes the overall frequencies.

Table 4.10: XRCC1 Arg399Gln genotype: Variant allele carriers compared with homozygous wild types

Case Control	XRCC399HetMutant		
	Carriers of variant allele	Homozygous wild-type	Total
Case	88	63	151
Control	92	67	159
Total	180	130	310

No significant associations were apparent (Fisher's test p = 1.000). Overall odds ratio for pancreatic cancer with the XRCC1 399 variant genotype was 1.017 (95% CI 0.648 to 1.598).

4.2.2 Gender sub-group analysis:

The influence of gender on the risk for pancreas cancer amongst the individual XRCC1 399 genotype was assessed and the frequencies are shown in Table 4.11

Table 4.11: XRCC1 Arg399Gln Genotypes - effect of gender

Gender		XRCC1 Arg399Gln		
		Carriers of variant allele	Homozygous wild-type	Total
Female	Case	42	25	73
	Control	48	35	94
	Total	90	60	167
Male	Case	46	38	91
	Control	44	32	82
	Total	90	70	173

No significant associations was discerned between the combined group and the wild-type genotype in the gender sub-groups (female Fisher's test p = 0.616, male Fisher's test p=0.750)

Estimating risk for female gender for pancreatic cancer; odds ratio = 1.125 (95% CI 0.633 to 2.369), while that for males was 0.880 (0.471 to 1.647).

4.2.3 Age sub-grouping

Table 4.12 shows data subdivided into age groupings – less than 65 years or above 65 years across genotype categories.

Table 4.12: XRCC1 ARg399Gln genotype - effect of age

		XRCC1 Arg399Gln		
		Carriers of variant allele	Homozygous wild-type	Total
≤65 years	Case	39	28	67
	Control	38	26	64
	Total	77	54	131
> 65 years	Case	49	35	84
	Control	54	41	95
	Total	103	76	179

No significant association is seen between age sub-groups and case-control status (≤ 65 years Fisher's test p=1.000, > 65 years Fisher's test p=0.880).

4.2.4 Smoking status

The effect of tobacco smoking was assessed by cross-tabulating smoking category and genotype. The frequencies across genotype sub-groups are shown in Table 4.13.

Table 4.13: XRCC1 Arg399Gln - Smoking status

		XRCC1 Arg399Gln		
		Carriers of variant allele	Homozygous Wild-type	Total
Present-smoker	Case	27	19	49
	Control	26	22	53
	Total	53	41	102
Past-smoker	Case	29	26	59
	Control	33	30	72
	Total	62	56	131
Non-smoker	Case	28	14	46
	Control	20	8	31
	Total	48	22	77

There were no significant associations seen between case/controls and genotype in all sub-groups based on smoking status [Fisher's test; present smokers p = 0.755; past smokers p = 0.602 and non- smokers p = 0.937].

Estimating risk for the polymorphism; for present smokers overall OR (95% CI) = 1.202 (0.531 to 2.721); for past smokers overall OR = 1.014 (0.491 to 2.092) and for non-smokers overall OR = 0.800 (0.282 to 2.266).

4.2.5 Smoking Intensity

The effect of tobacco smoking was also explored by grouping based on tobacco smoking intensity - Light smokers = ≤ 40 pack-years, heavy = > 40 pack-years cumulative tobacco exposure.

Table 4.14: XRCC1 ARg399Gln - effect of smoking intensity

		XRCC399HetMutant		
		Carriers of variant allele	Homozygous wild-type	Total
Light	Case	27	23	50
	Control	15	11	26
	Total	42	34	76
Heavy	Case	57	36	93
	Control	64	49	113
	Total	121	85	206

Cross tabulation reveals no association between genotype and case-control status amongst the smoking intensity sub-groups (Fisher's test, Light smokers p =0.811 and Heavy Smokers p = 0.569). Estimating the overall odds ratio for each smoking strata that for light smokers was 0.861 (95% CI 0.331 to 2.240) and that for heavy smokers 1.212 (95% CI 0.693 to 2.120).

4.2.6 Family history of malignancy

Performing family history of cancer sub-group analysis, in individuals who had valid and complete data for this history, we obtain Table 4.15. Incomplete history was obtained from n=92 (cases 28, controls n=64) and these individuals were excluded from this analysis.

Table 4.15: XRCC1 Arg280His - effect of family history of malignancy

		XRCC1 Arg399Gln		Total
		Carriers of variant allele	Homozygous wild-type	
FHP	Case	37	27	64
	Control	22	12	34
	Total	59	39	98
FHN	Case	33	26	59
	Control	37	24	61
	Total	70	50	120

No significant associations (Fisher's test) were seen amongst genotype and case-control status in the FHP (p=0.525) and FHN (p=0.711) sub-groups. Estimating risk for pancreas cancer amongst FHP, OR = 0.747 (0.316 to 1.768) and amongst FHN, OR = 0.823 (0.398 to 1.703).

4.2.7 Binary Logistic regression:

We performed binary logistic regression (n=206) to ascertain the effect of individual variables – age at recruitment, gender, smoking status, tobacco exposure, family history status and genotype – on risk for pancreatic cancer. Individuals with missing/incomplete data were excluded from the regression analysis.

The Hosmer Lemeshow test gave a X^2= 8.842 (p=0.356) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 36.091 p=0.0009. The model explained 21.7% (Nagelkerke R^2 = 0.217) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 69.4% of cases. Sensitivity was 87.8%, specificity was 42.2%, positive predictive value was 64.1% and negative predictive value was 70 %. Of the 5 predictor values only age and smoking status were significant – Table 4.16. Increasing age was associated with risk of pancreatic cancer, both present and past smokers were at increased risk but no specific risk was associated with the variant allele for XRCC1Arg399Gln. Age (the only continuous variable) and its logit transformation are not related significantly (p=0.154), therefore they are linearly related confirming that this logistic regression is valid.

Table 4.16: Logistic regression for XRCC1 Arg399Gln for susceptibility to pancreatic cancer

	B	Sig.	Exp(B)	95% C.I.for EXP(B)	
				Lower	Upper
Age	.889	.142	2.433	.743	7.966
Female Gender	-.438	.167	.645	.346	1.202
FHP	.288	.368	1.334	.712	2.498
Present Smoker	2.089	.000	8.077	3.293	19.814
Past Smoker	1.578	.001	4.843	1.979	11.853
XRCCArg399GlnHomozygous mutant	.347	.526	1.415	.484	4.141
XRCCArg399Gln Heterozygous	-.109	.748	.897	.462	1.741
Constant	-12.974	.073	.000		

Section 4.3 XRCCArg194Trp rs1799782

4.3.1: Overall association between genotype and case-control status.

Table 4.17 depicts the frequencies of the alleles of XRCC1 Arg194Trp in our study population. There were 28 individuals in whom this genotype could not be determined. For the population (n=312) the individual genotypes were in Hardy-Weinberg equilibrium, cases (X^2 = 0.59 , p=0.442) and controls (X^2 =0.29, p=0.590) with overall frequencies of major allele being 393 (92.69%) and 31 (7.31%). For cases the major allele frequency was 292 (94.16%) and minor allele frequency was 18 (5.81%) whilst amongst controls this was 301 (95.86) and 13 (4.14%).

Table 4.17: XRCC1 Arg194Trp genotype frequencies

Case Control		Frequency	Percent
Case	Missing	9	5.5
	Wild-type	137	83.5
	Heterozygous	18	11.0
	Total	164	100.0
Control	Missing	19	10.8
	Wild-type	144	81.8
	Heterozygous	13	7.4
	Total	176	100.0

The heterozygote genotype frequency amongst controls (13/312 = 4.16%) is close to that found in Caucasians [6.6%; (95% CI: 5.9–7.4)] (Schneider et al., 2008). No homozygous mutants were detected in either group. As shown in Table 4.18, no overall significant association was seen (Fisher's test p = 0.349) with overall odds ratio for pancreatic cancer 1.455 (95% CI 0.687 to 3.083).

Table 4.18: XRCC1 Arg194Trp genotypes - frequencies amongst cases and controls

	XRCC1 Arg194Trp		
	Carriers of variant allele	Homozygous Wild-type	Total
Case	18	137	155
Control	13	144	157
Total	31	281	312

4.3.2 Sub-group analysis: Gender

No association is seen on Fisher's test female (p=0.773 and male p=0.134). Overall odds ratio for female gender = 0.756 (95% CI 0.235 to 2.428) and that for males = 2.392 (0.811 to 7.058). These risks are not significant.

Table 4.19: XRCC1 Arg194Trp - effect of gender

		XRCC1 Arg194Trp		
		Carriers of variant allele	Homozygous Wild-type	Total
Female	Case	5	62	67
	Control	8	75	83
	Total	13	137	150
Male	Case	13	75	88
	Control	5	69	74
	Total	18	144	162

4.3.3 Age sub-groupings:

With 65 years as a cut-off age subgroup analysis was performed and the data is shown in Table 4.20 and no significant association is seen on Fisher's test (≤ 65 years p=0.103, > 65 years p=0.797).

Table 4-20: XRCC1 Arg194Trp - effect of age

		XRCC1 Arg194Trp		
		Carriers of variant allele	Homozygous wild-type	Total
≤65 years	Case	11	59	70
	Control	4	60	64
	Total	15	119	134
> 65 years	Case	7	78	85
	Control	9	84	93
	Total	16	162	178

Calculating odds ratio for pancreatic cancer for individuals below 65 years of age we obtain 2.79 (95% CI 0.84 to 9.28) while that for those above 65 years of age is 0.83 (0.29 to 2.35). These risk estimates are not significant. Although these risk estimates are not significant, the presence of the variant allele amongst the younger age group appears to suggest an increased predisposition to pancreatic cancer as compared to the older group.

4.3.4 Analysis by smoking status

The impact of tobacco smoking and individual genotypes on risk for pancreatic cancer was explored and the data is shown in Table 4.21.

Table 4.21: XRCC1 ARg194Trp - effect of smoking status

		XRCC1 Arg194Trp		
		Carriers of variant allele	Homozygous Wild-type	Total
Present-smoker	Case	10	70	80
	Control	6	55	61
	Total	16	125	141
Past-smoker	Case	7	50	57
	Control	2	35	37
	Total	9	85	94
Non-smoker	Case	1	7	8
	Control	4	37	41
	Total	5	44	49

No association of present, past and non-smoking statuses with genotype was found for disease risk (Fishers test p = 0.790, 0.474, 1.000 respectively). Estimating risk for pancreatic cancer in the various tobacco smoking exposure groups, the overall odds ratio (95% CI) for present smokers was 1.310 (0.448 to 3.825) for past smokers 2.450 (0.480 to 12.501) and non-smokers 0.147 (0.015 to 1.374) all of which were not significant.

4.3.5 Smoking intensity

The impact of tobacco smoking and genotype for XRCC1 194 was also assessed by subdividing individuals based on tobacco smoking intensity and the frequencies in the groupings (light = ≤40 pack-years and heavy = >40 pack-years) as shown in Table 4.22.

Table 4.22: XRCC1 Arg194Gln - effect of tobacco smoking intensity

		XRCC1 Arg194Trp		
		Carriers of variant allele	Homozygous Wild-type	Total
Light	Case	4	47	51
	Control	2	23	25
	Total	6	70	76
Heavy	Case	13	81	94
	Control	10	104	114
	Total	23	185	208

No significant associations were seen for case-control status amongst light (Fishers test p= 1.000) and heavy smokers (Fishers test p=0.272). Odds ratio (95% CI) for pancreatic cancer for light smokers was 0.979 (0.167 to 5.741) and that for heavy smokers was 1.669 (0.696 to 4.000) which are not significant.

4.3.6 Analysis for family history sub groups:

Table 4.23 depicts the frequencies of the individual genotypes for XRCC1 194 and history of malignancy in the first-degree relatives of the study population. Full family history was available for 205 individuals.

Table 4.23: XRCC1 Arg194Trp - effect of family history of malignancy

Family history of cancer		Case Control		
		Case	Control	Total
FHP	Homozygous Wild-type	55	32	87
	Carriers of variant allele	11	1	12
	Total	66	33	99
FHN	Homozygous Wild-type	54	42	96
	Carriers of variant allele	6	4	10
	Total	60	46	106

No significant associations are seen for case-control status among the family history groupings (FHP, Fishers test p=0.056 and FHN, Fishers test p=1.000).

4.3.7 Logistic regression model:

Logistic regression was performed excluding incomplete and missing values (amongst genotype, age, gender, family history and smoking status) to ascertain the impact of these variables on the likelihood of developing pancreatic ductal adenocarcinoma. The total numbers of cases included was 207 (Table 4.24). The Hosmer Lemeshow test gave a X^2= 5.448 (p=0.709) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 42.487, p=0.000004. Age (the only continuous variable) and its logit transformation were not related significantly (p=0.868), confirming that this logistic regression is valid.

The model explained 25.1 % (Nagelkerke R^2 = 0.251) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 72 % of cases. Sensitivity was 91.1%, specificity was 43.4 %, positive predictive value was 70.6% and negative predictive value was 76.59 %. Of the 5 predictor values only age and smoking status were significant – Table 4.24. Increasing age was associated with risk of pancreatic cancer, both present and past smokers were at increased risk but no specific risk was associated with the variant allele for XRCC1 Arg148Trp genotype.

Table 4.24: Logistic regression on XRCC1 Arg194Trp genotype and relationship to pancreatic cancer susceptibility

	B	Sig.	Odds ratio	95% C.I. for Odds ratio	
				Lower	Upper
Age	.751	.251	2.119	.588	7.636
Female Gender	-.553	.087	.575	.306	1.083
FHP	.284	.379	1.328	.706	2.498
Present Smokers	2.236	.000	9.359	3.676	23.829
Past Smokers	1.818	.000	6.157	2.416	15.695
XrCC1 Arg194Trp Het	-.349	.505	.706	.253	1.968
Constant	-10.949	.162	.000		

Section 4.4 XRCC 1 Arg280His

4.4.1 Overall association between genotype and case-control status

The frequencies of the individual genotypes are depicted in Table 4.25. Both the cases (X^2 = 0.93, p=0.334) and controls (X^2 = 0.37, p=0.543) were in Hardy-Weinberg equilibrium and the overall allele frequencies were major allele 593 (94.42%) and minor allele 35 (5.57%). The allele frequencies amongst cases were major allele 294 (94.83%) and minor allele 16 (5.16%), amongst controls they were 299 (94.09%) and 19 (5.97%) respectively.

Table 4.25: XRCC1 Arg280His genotype frequencies amongst cases and controls

		Case Control		
		Case	Control	Total
XRCC1 Arg280His	Missing	9	17	26
	Homozygous Mutant	1	1	2
	Heterozygote	14	17	31
	Homozygous wild-type	140	141	281
	Total	164	176	340

Combining heterozygotes and mutants into a combined group and ignoring missing values, we obtain Table 4.26.

Table 4.26: XRCC1 Arg280His - variant allele risk for pancreatic cancer

	XRCC1 Arg280His		
	Carriers of variant allele	Homozygous wild-type	Total
Case	15	140	156
Control	18	141	156
Total	33	281	314

No significant association between case/control status and genotype was seen (Fisher's test p=0.714). Overall odds ratio for the variant allele for pancreatic cancer was 0.839 (95% CI 0.407 to 1.731).

4.4.2 Gender subgroup analysis:

The impact of gender and genotype for XRCC1 Arg280His on risk for pancreatic cancer was explored, and the data is shown in Table 4.27.

Table 4.27: XRCC1 Arg280His - effect of gender

Gender		XRCC1 Arg280His		
		Carriers of variant allele	Homozygous wild-type	Total
Female	Case	7	64	71
	Control	9	74	83
	Total	16	138	154
Male	Case	8	76	84
	Control	9	67	76
	Total	17	143	160

There were no significant associations between genotype and case/control status in either gender subgroups (Fisher's test; Female gender: p = 1.000; Male gender: 0.798). Overall odds ratio for female gender for pancreatic cancer was 0.899 (95% CI 0.317 to 2.552) while that for male gender was 0.784 (95% CI 0.286 to 2.146).

4.4.3 Age category subgrouping

In order to ascertain if there were any particular age groups, which were associated with the mutant allele, we grouped our patients with 65 years as a cut off point into 2 groups The data re shown in Table 4.28.

Table 4.28: XRCC1 Arg280His - effect of age

		Case Control		
		Case	Control	Total
≤ 65 years	Carriers of variant allele	8	8	16
	Homozygous wild-type	60	56	116
	Total	68	64	132
>65 years	Carriers of variant allele	7	10	17
	Homozygous wild-type	80	85	165
	Total	87	95	182

No significant associations were seen in either the up to 65 years group (Fishers test p= 1.000) or in the above 65 years group (Fisher's test p = 0.618) between genotype and case/control status. The less than 65 years age group demonstrated an odds ratio for pancreatic cancer of 0.933 (95% CI 0.328 to 2.655) while that for those above 65 years was 0.744 (95% CI 0.270 to 2.048).

4.4.4 Smoking exposure sub groups

The influence of tobacco smoking and genotype for XRCC1 Arg280His on risk for pancreas cancer was assessed and the data is shown in Table 4.29. Incomplete data was found in 28 samples and these were excluded from this analysis.

Table 4.29: XRCC1 ARg280His - effect of smoking status

		Case	Control	Total
Present-smoker	Carriers of variant allele	12	8	20
	Homozygous wild-type	69	53	122
	Total	81	61	142
Past-smoker	Carriers of variant allele	2	3	5
	Homozygous wild-type	54	35	89
	Total	56	38	94
Non-smoker	Carriers of variant allele	0	2	2
	Homozygous wild-type	10	38	48
	Total	10	40	50

No significant associations were demonstrated for the different smoking statuses (Fisher's test; present smokers p = 0.812, past smokers p = 0.390, non-smokers p=1.000). Odds ratios (95% CI) for the variant genotype amongst the respective smoking statuses were 1.152 (0.440 to 3.020), 0.432 (0.069 to 2.718) and 1.283 (1.09 to 1.460).

4.4.5 Smoking intensity

The influence of tobacco smoking was explored by assessing intensity of smoking and genotype for XRCC1 ARg280His and the data is shown in Table 4.30.

Table 4.30: XRCC1 Arg280His - effect of intensity of tobacco smoking

		Case Control		
		Case	Control	Total
Light (≤40 pkyrs)	Carriers of variant allele	4	4	8
	Homozygous wild-type	47	22	69
	Total	51	26	77
Heavy (>40 pkyrs)	Carriers of variant allele	10	9	19
	Homozygous wild-type	86	104	190
	Total	96	113	209

No significant associations were seen (Fishers test; light smokers p = 0.431, heavy smokers = 0.631). Odds ratio for pancreatic cancer for light smokers = 0.468 (95% CI 0.107 to 2.047) and for heavy smokers = 1.344 (95% CI 0.522 to 3.456).

4.4.6 Family history sub groupings

The impact of a family history of malignancy and genotype for XRCC1 Arg280His and risk for pancreatic cancer was explored and the frequencies of the data are shown in Table 4.31. A family history was unavailable in n = 92 individuals and they were excluded from this analysis.

Table 4.31: XRCC1 Arg280His - effect of family history of malignancy

		Case Control		
		Case	Control	Total
FHP	Carriers of variant allele	6	2	8
	Homozygous Wild-type	62	32	94
	Total	68	34	102
FHN	Carriers of variant allele	8	6	14
	Homozygous Wild-type	51	55	106
	Total	59	61	120

No significant association was seen between genotype and family history of cancer for pancreatic cancer risk (Fishers test; FHP p= 0.715; FHN p = 0.579). Odds ratio for pancreatic cancer risk for FHP was 1.548 (95% CI 0.296 to 8.113) and that for FHN was 1.438 (95% CI 0.467 to 4.429).

4.4.7 Logistic regression

Logistic regression was performed excluding incomplete and missing values (amongst genotype, age, gender, family history and smoking status) to ascertain the impact of these variables on the likelihood of developing pancreatic ductal adenocarcinoma. The total numbers of cases included was 210 (Table 4.32). The Hosmer Lemeshow test gave a X^2= 5.640 (p=0.687) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 37.317 p=0.0000005. Age (continuous variable) and its logit transformation are not associated significantly (p=0.206), confirming that this logistic regression is valid.

The model explained 22 % (Nagelkerke R^2 = 0.220) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 70.5% of cases. Sensitivity was 88.2%, specificity was 43.4%, positive predictive value was 70.4% and negative predictive value was 70.58 %. Of the 6 predictor values only age and smoking status were significant – Table 4.32. Increasing age was associated with risk of pancreatic cancer, both present and past smokers were at increased risk but no specific risk was associated with the variant allele for XRCC1Arg399Gln.

Table 4.32: Logistic regression on XRCC1 Arg280His and relationship to pancreatic cancer susceptibility

	B	Sig.	Odds ratio	95% C.I.for Odds ratio	
				Lower	Upper
Age	.029	.027	1.030	1.003	1.057
Female Gender	-.443	.156	.642	.348	1.185
FHP	.407	.193	1.502	.814	2.770
Present Smoker	2.049	.000	7.760	3.217	18.719
Past Smoker	1.530	.001	4.619	1.913	11.150
XRCC1 ARg280His Het	.648	.296	1.912	.567	6.448
Constant	-2.953	.002	.052		

Section 4.5: APE1 Asp148Gln rs1130409

Table 4.33 shows the frequency of the individual genotypes for APEAsp148Gln. There were 30 individuals with missing genotype data. The population under study was in Hardy-Weinberg equilibrium; cases (X^2 =0.38, p=0.537), controls (X^2=0.41, p=0.522) and the overall frequencies for the major allele 299 (48.23%) and minor allele 321 (51.77%). The allele frequencies amongst cases were major allele 144 (47.37%) and minor allele 160 (52.63%), amongst controls this was 155 (49.05%) and 161 (50.95%) respectively.

Table 4.33: APE1 Asp148Gln overall frequencies

APE1Asp148Gln genotype	Case Control		
	Case	Control	Total
Missing	12	18	30
Homozygous wild-type	36	36	72
Heterozygous	72	83	155
Homozygous Mutant	44	39	83
Total	164	176	340

4.5.1 Overall associations between genotype and case-control status

As shown in Table 4.34, when heterozygotes and homozygous mutants are combined group as carriers of the variant allele, no significant associations are seen (Fishers test p=0.893); overall odds ratio for pancreatic cancer 0.951(95% CI 0.561 to 1.611).

Table 4.34: APE1 Asp148Gln genotype frequencies amongst cases and controls

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous wild-type	Total
Case Control	Case	116	36	152
	Control	122	36	158
	Total	238	72	310

4.5.2 Gender subgroup and risk

As shown in Table 4.35, there were no significant differences (Fisher's test) between genotype groupings for the female (p= 0.571) and male gender (p=0.333). Amongst females; odds ratio for pancreatic cancer for pancreas cancer was 1.329 (95% CI 0.628 to 2.810) and amongst males it was 0.639 (95% CI 0.294 to 1.387).

Table 4.35: Ape1 Asp148Gln - gender sub-grouping

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous Wild-type	Total
Female	Case	52	15	67
	Control	60	23	83
	Total	112	38	150
Male	Case	64	21	85
	Control	62	13	75
	Total	126	34	160

4.5.3 Age sub-group and risk

We divided our patients into a less than 65 years and above 65 years to assess risk in age sub-groups and the frequencies are shown in Table 4.36.

Table 4.36: APE1 Asp148Gln - age sub-grouping

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous Wild-type	Total
≤65 yrs	Case	46	19	65
	Control	48	17	65
	Total	94	36	130
>65 yrs	Case	70	17	87
	Control	74	19	93
	Total	144	36	180

There was no significant association between genotype and pancreas cancer status in either age-group (Fisher's test; ≤ 65 years; p=0.844, > 65 years; p=1.000). Odds ratio for pancreatic cancer was 0.857 (95% CI 0.397 to 1.850) in the younger age group and 1.057 (95% CI 0.509 to 2.197) in the older age group.

4.5.4 Smoking subgroups and risk

There were 28 (8 cases and 20 controls) individuals with incomplete smoking exposure information and these have been excluded from further analysis. Table 4.37 shows the frequencies of the individual sub-groups.

Table 4.37: APE1 Asp148Gln - smoking status groups

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous wild-type	Total
Present-smoker	Case	59	21	80
	Control	51	10	61
	Total	110	31	141
Past-smoker	Case	41	13	54
	Control	26	11	37
	Total	67	24	91
Non-smoker	Case	9	1	10
	Control	33	7	40
	Total	42	8	50

There was no significant association in the individual smoking exposure sub-groups between genotype and case/control status (Fisher's test): present smokers p = 0.218, past smokers p = 0.630 and non-smokers p = 1.000. The odds ratio for pancreatic cancer risk with this polymorphism for the individual smoking statuses was as follows: present smokers 0.551 (95% CI 0.238 to 1.278), past-smokers 1.334 (95% CI 0.521 to 3.420) and non-smokers 1.909 (95% CI 0.207 to 17.598).

4.5.5 Smoking intensity

As shown in Table 4.38, when the risk for light (up to 40 pack-years) and heavy (more than 40 pack-years) smoker's was examined, no significant association was found (Fisher's test; light smokers p = 1.000, heavy smokers p = 0.597).

Table 4.38: APE1 Asp148Gln - smoking intensity sub-groups

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous wild-type	Total
Light	Case	35	15	50
	Control	18	8	26
	Total	53	23	76
Heavy	Case	74	20	94
	Control	92	20	112
	Total	166	40	206

The odds ratio with this polymorphism for pancreatic cancer for light smokers was 1.037 (95% CI 0.370 to 2.903) and that for heavy smokers was 0.804 (95% CI 0.403 to 1.606).

4.5.6 Family history sub groups and risk

A total of n=102 (cases 29, controls 63) individuals did not have a full family history and these were excluded from this analysis. Table 4.39 shows the frequencies of the individual sub-groups.

Table 4.39: APE1 Asp148Gln - family history of cancer sub-groups

		APE1 Asp148Gln		
		Carriers of variant allele	Homozygous wild-type	Total
FHP	Case	53	11	64
	Control	26	8	34
	Total	79	19	98
FHN	Case	40	19	59
	Control	47	14	61
	Total	87	33	120

There was no significant association between genotype and case/control status amongst those who had a history of cancer in their family (FHP, Fisher's test p = 0.592) and amongst those who did not have this history (FHN, Fisher's test p=0.308). Estimating risks, overall odds ratio for pancreatic cancer with this polymorphism for FHP = 1.483 (95% CI 0.532 to 4.130) and that for FHN = 0.526 (95% CI 0.211 to 1.310).

4.5.7 Logistic regression:

Logistic regression was performed excluding incomplete and missing values (amongst genotype, age, gender, family history and smoking status) to ascertain the impact of these variables on the likelihood of developing pancreatic ductal adenocarcinoma. The total numbers of cases included was 229 (Table 4.40). The Hosmer Lemeshow test gave a X^2= 5.148 (p=0.742) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 39.19, p=0.0000002. Age (the only continuous variable) and its logit transformation are not related significantly (p=0.871), confirming that this logistic regression is valid.

The model explained 23.4 % (Nagelkerke R^2 = 0.234) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 69.4 % of cases. Sensitivity was 86.2%, specificity was 44.6 %, positive predictive value was 69.73 % and negative predictive value was 68.51 %. Of the 5 predictor values only age and smoking status were significant – Table 4.40. Increasing age was associated with risk of pancreatic cancer, both present and past smokers were at increased risk but no specific risk was associated with the variant allele for APE Asp148Gln genotype.

Table 4.40: Logistic regression on APE1 Asp148Gln and relationship to pancreatic cancer susceptibility

	B	Sig.	Odds ratio	95% C.I. for Oddds ratio	
				Lower	Upper
Age	.034	.013	1.035	1.007	1.063
Female Gender	-.564	.076	.569	.305	1.061
FHP	.262	.410	1.300	.697	2.427
Present Smoker	2.085	.000	8.041	3.340	19.363
Past Smoker	1.526	.001	4.598	1.889	11.188
APE Asp148Gln variant allele	-.431	.270	.650	.302	1.397
Constant	-2.844	.004	.058		

Section 4.6 Logistic regression including all genotypes

A model which included variables known to influence risk for pancreas cancer like age, gender, family history of cancer, tobacco smoking and the genotypes under investigation which had been analysed in our study population was tested to ascertain the combined effects of these factors. For this model we only included individuals who had valid data with regard to the variables incorporated. There were 192 individuals in total.

The Hosmer Lemeshow test gave a X^2= 7.128 (p=0.523) indicating a good fit of the data to the model. Also the model was statistically significant X^2 = 40.85, p=0.000005. Age (the only continuous variable) and its logit transformation are not related significantly (p=0.326), confirming that this logistic regression is valid.

The model explained 25.3 % (Nagelkerke R^2 = 0.253) of variance in the development of pancreatic ductal adenocarcinoma and correctly classified 70.3 % of cases. Sensitivity was 87.7%, specificity was 44.9 %, positive predictive value was 69.93 % and negative predictive value was 71.42 %. Of the 9 predictor values only age and smoking status were significant – Table 4.41.

Table 4.41: Logistic regression including all studied genotypes and risk for pancreatic cancer

	B	Sig.	Odds ratio	95% C.I. for Odds ratio	
				Lower	Upper
Age	.031	.046	1.032	1.001	1.064
Female Gender	-.718	.032	.488	.253	.940
FHP	.219	.521	1.245	.638	2.428
Present Smoker	2.144	.000	8.531	3.198	22.759
Past Smoker	1.769	.000	5.862	2.223	15.460
APE1Asp148Gln variant allele	-.136	.774	.873	.345	2.207
hOGG1 (rs2072668) variant allele	1.242	.222	3.461	.472	25.388
XRCC1Arg280His variant allele	.708	.275	2.031	.569	7.253
XRCC1Arg399Gln variant allele	-.011	.986	.989	.304	3.220
XRCC1Arg194Trp variant allele	-.208	.699	.812	.283	2.329
Constant	-3.915	.018	.020		

CHAPTER 5: MAIN FINDINGS AND DISCUSSION

Section 5.1: Main findings

The main findings from this study are

1. Tobacco smoking is a risk factor for pancreatic cancer

    a. The risk for an ever smoker (current and ex) for the development of pancreatic cancer is nearly 3 times that of a non-smoker [OR 3.01 (95% CI 1.73 to 5.24)].

    b. Amongst pancreatic cancer cases, there was a significantly decreased cumulative tobacco exposure in the caFDR+ group (p=0.023) as compared to the caFDR- group: the overall cumulative pack-years of smoking was 32.79 (24.77) in the caFDR+ versus 45.69 (24.56) in the caFDR- group.

2. No significant major overall risks were associated with the individual genotypes for the various BER genes that were typed both individually and when all genotypes were grouped into a single model (Binary logistic regression).
3. On multi-variate analysis which included variables known to influence risk for pancreas cancer – age, tobacco smoking and the factors under consideration – family history of malignancy and the individual genotypes under investigation;
    a. Tobacco smoking showed an increased risk – present smokers exhibited an OR = 8.531 (3.198 to 22.759) and past smokers demonstrated an OR = 5.862 (2.223 to 15.460).
    b. Increasing age appeared to increase risk [OR (95% CI) = 1.032 (1.001 to 1.064)] but this could be a result of the design of the study rather than a significant finding.

Section 5.2 Discussion:

In this prospective case-control study, we have included 164 cases of pancreatic ductal adenocarcinoma and compared them with 176 hospital-based controls. The vast majority of cases included (74%) were histologically/cytologically diagnosed, 42 (26%) were deemed to have pancreatic ductal adenocarcinoma on the basis of a combination of the presence of a progressive pancreatic mass (on serial imaging and or rising tumour markers in the presence of a compatible history). Logistical and research funding limitations meant that a population based control group could not be recruited and therefore a hospital based group was decided upon. Smoking exposure and family history data was recorded by personal interview of the subjects by one single investigator and data recorded concurrently on to an electronic database. Several limitations of this data collection are important to note – no usable alcohol exposure data was collected; family history data was deficient in that ages of onset of cancer in the FDR's, the total number of FDR's in the family and their current health status were not recorded. Therefore person life-years of risk have not been able to be calculated. Due to limitations of available time enough prospective controls could not be recruited and historical controls n=50 from a former study were included. Although demographic and tobacco smoking data for these historical controls was available detailed tobacco exposure data and family history of cancer information was not obtainable. Therefore the analysis of tobacco smoking, family history of malignancy and their interaction (Chapter 3) as risks for pancreatic cancer data suffer from a preponderance of cases (n=134) as compared to controls (119). All genotyping data included 164 cases and 176 controls.

5.2.1 Family history and tobacco exposure

It has been observed from epidemiological studies that the first-degree relatives of sporadic cancer patients have a 2 to 3-fold higher risk of developing cancer at the same site and this has also been described for pancreatic cancer but only in retrospective studies (Del Chiaro et al., 2007, Ghadirian et al., 2002, McWilliams et al., 2005b). In a prospective observational study Teresmette et al (Tersmette et al., 2001) showed that relatives of familial pancreatic cancer patients had a higher risk of developing pancreatic cancer. The PCs occurred in relatives who were usually living in different communities during their adult lives, and the cancers occurred in the sixth or greater decade of life and therefore the suggestion was that this increased risk had a genetic basis rather than the result of shared environmental exposure.

Familial clustering observed in certain sporadic cancers without obvious Mendelian inheritance suggests that there is a genetic component in addition to environmental factors (Li, 1990). This could be explained on the basis that the family members with the similar genetic background are exposed to the differing environment and that this leads to the phenotypic manifestation of the disease. Analysis of genetic risk of cancer has shown that most non-hereditary, sporadic cancers develop in genetically predisposed individuals, this predisposition being the result of several low penetrant genes (Imyanitov et al., 2004, Houlston and Peto, 2004). These poorly (low) penetrant genes, which by themselves have small relative risks, by virtue of being common in the population, may have large population attributable risks (Del Chiaro et al., 2007).

The interplay of environmental and genetic factors appears to play a critical role in the development of pancreatic cancer and this has been well described for its familial form (Brentnall, 2005). It is reasonable to suppose that the remaining cases of sporadic adenocarcinoma of pancreas, which form the majority, are due to gene-environment

interaction (GEI). These have been poorly characterized and therefore the majority of sporadic pancreatic cancers have been considered to have no identifiable cause and therefore no high-risk groups are identifiable.

In our prospective hospital-based case-control study we have seen that pancreatic cancer patients smoked more than our control group and an ever-smoker individual had a 3-times higher risk for the development of pancreatic cancer than a non-smoker. These are well-recognised findings. More importantly a family history of malignancy in first-degree relatives appeared to decrease the amount of tobacco exposure (as measured by pack-years) required for the development of pancreatic cancer. The earlier onset of the disease was however not related to FDR status. In addition, however, there were other results; smokers on average developed the cancer about 6-7 years earlier than non-smokers, which was independent of a family history of malignancy. This has been previously described on the basis of WHO cancer mortality data and SEER cancer incidence data (Raimondi et al., 2007). However the findings from our study suffer from a limitation of non-availability of complete family history data, as mentioned above and therefore this finding has to be ignored.

It is accepted that familial pancreatic cancer appears to develop at an earlier age as compared to its sporadic counterpart and tobacco exposure is the most important factor influencing the penetrance of the FPC gene (Brentnall, 2005). Smokers in FPC (James et al., 2004, Rulyak et al., 2003c) and in hereditary pancreatic cancer syndromes, specifically hereditary pancreatitis patients (Lowenfels et al., 2001) develop the disease about 10 years earlier demonstrating the interaction between an inherited susceptibility to cancer and an environmental carcinogen. A recent report has described gene-environment interaction in a study of cases only, although the sample size was large (Yeo et al., 2009). From our study it appears that smokers who have a family history of

cancer develop the disease at a lower level of exposure. This might be due to continued or faster accumulation of genotoxic mutations secondary to a variety of factors, one of which might be an inefficient DNA repair mechanism. Other genetic and environmental factors might play a role and this will need further elucidating. For example, a recent report has shown an earlier age of onset of pancreatic cancer in those who had a high BMI during their teen and younger years (Li et al., 2009).

The groups of index cases and controls with and without a family history of cancer were comparable given their similar age distribution and gender distribution. We have obtained history of cancer in FDR from index cases and controls and it is known that such information is reliable and accurate especially with regard to FDRs (Murff et al., 2004). The reliability of information obtained, however, decreases with regard to other relatives (Ziogas and Anton-Culver, 2003, Parent et al., 1997) and we have therefore restricted our study to data on first-degree relatives. It has been suggested that, if anything there is under reporting of family history of cancer especially with regard to colorectal neoplasms (Mitchell et al., 2004). Other details of the illness in the FDR such as age of onset (of the cancer in the relative obtained from the index case individual) is unreliable especially in older probands and we have therefore not utilised such data in our study (Parent et al., 1997). We have not performed genetic analysis in this group of patients to confirm that they are not familial cancers as most familial pancreatic cancers are not due to known mutations. It is likely that our patients represent sporadic malignancies due to the fact that there was no difference in the mean (SD) of the age at diagnosis of the index cases in the FDR+ and FDR- groups [65.93 (10.67) and 64.57 (12.38) years].

In the presence of a family history of any malignancy, irrespective of smoking, the risk for pancreatic cancer is double [OR 1.98 (95% CI: 1.15-3.38)]. In individuals with a

first-degree family history of malignancy (i.e. increased susceptibility), a decreased dose of an environmental carcinogen is sufficient to cause cancer [cumulative tobacco exposure in FDR+ = 32.79 (24.77) vs. FDR- = 45.69 (24.56) (Mann-Whitney test p=0.023) Table 3.5]. However due to non-availability of date on age of onset of malignancy in the FDR and the total number of FDR's in the family, person-years of risk and standardized incidence ratios could not be calculated. Just under 2/3rds of FDR+ index cases (n=51; 65%) had just a single first-degree relative with malignancy. In the FDR+ group decreased tobacco exposure was required for the development of adenocarcinoma of the pancreas but this did not depend upon the number of relatives with malignancy, as the FDR>1 group did not demonstrate a significantly decreased cumulative tobacco exposure. It is well accepted that a family history of cancer is a risk factor for most cancer types. With respect to adenocarcinoma of the pancreas, a recent meta-analysis of seven case-control and two cohort studies involving 6,568 pancreatic adenocarcinoma cases concluded that a family history of adenocarcinoma of the pancreas conferred double the risk [1.80 (95% CI: 1.48-2.12)] for the disease in individuals with such a history compared to those without (Permuth-Wey and Egan, 2008). A recent cohort study from the PanScan consortium (Jacobs et al.) and prospective follow-up of participants of Cancer Prevention Study-II (Jacobs et al., 2009) have suggested an association between family history of various cancers especially prostate cancer and pancreatic cancer.

It is possible that the decreased tobacco dose demonstrated in the caFDR+ group is due to a genetic or other environmental factor which potentiates the genotoxic effect of tobacco derived carcinogen by either impairing the processing of tobacco derived carcinogen into inactive metabolites or causing the inefficient or incomplete repair of genetic damage induced by it.

5.2.2 SNP analysis

In our cohort no overall statistically significant risk for hOGG1 (rs2072668), XRCC1 Arg194Trp, XRCC1 Arg280His, XRCC1 Arg399Gln, APE Asp148Gln genotypes and pancreatic cancer was detected.

hOGG1

The hOGG1 genotype studied did not confer any risk for pancreatic cancer. This is interesting as the 8-oxoguanine DNA glycosylase (OGG1) gene is a BER gene that removes oxidative DNA lesions (David et al., 2007) and it would appear that CC/CG genotype, combined with environmental exposure could increase susceptibility to pancreatic cancer. However although some functional studies suggest reduced repair function with variant alleles in hOGG1, the evidence is generally inconclusive (Weiss et al., 2005b). However recently it has been shown that the pancreatic adenocarcinoma cell line BxPC-3 is deficient in the repair of 8-OH-Gua relative to the non-malignant cell line AG11134. The deficient repair of 8-OH-Gua was shown to be associated with defects in expression levels and activity of hOGG1 since BxPC-3 cells exhibited undetectable level of hOGG1 and had severely down regulated hOGG1 mRNA(Nyaga et al., 2008). There is some clinical evidence in support of this finding from a hospital case–control study conducted at MD Anderson Centre in the United States, Li et al noted not only an increased risk for pancreas cancer with the CC/CG genotype, but also significantly reduced overall survival in patients with the OGG1 C315G (rs1052133) GG homozygous variant genotype (Li et al., 2007a).

The reason why this finding was not duplicated in our study might be due to the small numbers of individuals. The deficient oxidative repair capacity of the variant gene might be more apparent amongst smokers, as seen in the current study amongst current

smokers the risk for the variant genotype and pancreas cancer was 1.342 (0.656 to 2.747) as compared to non-smokers [0.317 (0.071 to 1.407)].

Li etal also reported a weak interaction of the OGG1 CC/CG genotype with diabetes in pancreatic cancer(Li et al., 2007b). This group also found that XRCC2 Arg188His polymorphisms may be genetic modifiers for smoking-related pancreatic cancer(Jiao et al., 2008a).

There is some evidence for the role of other genotype polymorphisms in pancreatic carcinogenesis too from other molecular epidemiological studies: the presence of XRCC2 Arg188His polymorphism modulates risk for pancreatic cancer amongst smokers (Jiao et al., 2008b); XPD gene polymorphisms – exon 10 Asp(312)Asn and exon 23 Lys(751)Gln polymorphisms influence risk for smoking associated adenocarcinoma of the pancreas (Jiao et al., 2007) and deletion polymorphism in GSTT1 is associated with an increased risk of adenocarcinoma of the pancreas amongst Caucasians (Duell et al., 2002a).

XRCC1 Arg399Gln

In our study genotype for XRCC1 Arg399Gln and its alleles did not demonstrate a significant risk for pancreatic cancer.

On the basis of 6,120 lung cancer cases and 6,895 controls from 13 studies, skin malignancies (3 studies) and breast cancer (11 studies) no association between the 399Gln allele and cancer risk was seen. However non-significant decreased risk of upper aero-digestive tract cancer and non-significant increased risk of bladder carcinoma for heterozygotes and mutants was detected. When risk was stratified by tobacco smoking for tobacco related cancers, the presence of the 399Gln allele seemed

to be associated with an increased risk of tobacco-related cancers among light smokers (OR = 1.20, 95 % CI: 1.03-1.40), whereas it was associated with a decreased risk among heavy smokers (OR = 0.81, 95 % CI: 0.64-1.04), with the effect being more prominent among carriers of the Gln/Gln genotype (OR = 0.71, 95 % CI: 0.51, 0.99) (Hung et al., 2005a).

A meta-analysis by Hung RJ et al (Hung et al., 2005b) observed a modification of the effect of XRCC1 399/399Gln genotype on risk for pancreas cancer by tobacco smoking. The mutant allele 399Gln is associated with higher mutagen sensitivity and DNA adduct levels (Wang et al., 2003, Lunn et al., 1999). One mechanism suggested is that increased levels of DNA damage from heavy smoking in Gln/Gln carriers may cause apoptosis during cell division rather than development of a clone of cancer cells. Another suggestion is that DNA damage causes up-regulation of DNA repair capacity and more efficient repair of nucleotide lesions. There is some evidence from some studies in support of these hypotheses (Wang et al., 2003, Nelson et al., 2002, van Zeeland et al., 1999).

In a study of 309 cases of pancreatic adenocarcinoma and 964 population based controls, relative to never active or passive smokers with the Arg/Arg genotype, the age- and race-adjusted ORs (95% CIs) for heavy smoking (≥41 pack-years) were, for Gln/Gln or Arg/Gln genotypes in women 7.0 (2.4, 21) and men 2.4 (1.1, 5.0)] and for the Arg/Arg genotype in women 2.2 (0.73, 6.4) and men 1.5 (0.68, 3.2)] (Duell et al., 2002b)**.** There was no overall association between genotype for XRCC1 Arg399Gln and pancreatic cancer. The suggestion here is that there is interaction between XRCC1 399Gln and smoking that was stronger among women than men. However, these findings need to be confirmed in other studies, as the number of study subjects was small in the analysis exploring gene–environment interaction.

These results were not duplicated in our study population. Again our study population is very small and the numbers especially on gender and tobacco exposure sub-group analysis amongst the individual genotype categories of XRCC1 399 are not large enough to tease out these risks estimated on large population studies. In a hospital based study involving 101 cases and 307 controls very similar results to our own were found – non-significant decrease in risk for those carrying Gln/Gln genotype at XRCC1 Arg399Gln site (OR 0.64, 95% CI 0.21 - 1.66, P = 0.30) compared with those having Arg/Arg genotypes, so no association between XRCC1 Arg399Gln genotype and pancreatic cancer risk (Wang et al., 2006a) was seen.

XRCC1 Arg194Trp

XRCC1 Arg194TRP did not demonstrate an overall association with development of pancreatic cancer.

Previously published results have suggested either no association (Misra et al., 2003) or a protective effect on tobacco related cancers (Ratnasinghe et al., 2003) (Hung et al., 2005a). Mutagen sensitivity assays have suggested that the XRCC1 Arg194Trp variant allele has a protective effect on bleomycoin and BPDE-induced chromosomal damage i.e. less number of chromosomal breaks per cell on bleomycion and BPDE assays (Wang et al., 2003). There was also no significant difference in the sister chromatid exchange assay prior to and after treatment with tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), from volunteers with the variant XRCC1 Arg194Trp genotype (Abdel-Rahman and El-Zein, 2000). Studies in Chinese subjects in whom the incidence of the heterozygous genotype is high [31.22% (95% CI) 29.6 to 32.8)] have suggested an increase in gastric cancer risk for heterozygotes for the XRCC1 194 polymorphism. In addition, this study also examined the XRCC1 399

polymorphism (the incidence of which is lower than Caucasian population; 24.6 vs 34.1%) (Shen et al., 2000) but the risk was not significant. In a Chinese study which explored risk for XRCC 399 (vide supra), no significant risks were associated with XRCC Arg194Trp genotype and pancreatic cancer (Wang et al., 2006a).

An interesting finding from our study is that there is a non-significant trend towards an increased risk for the variant genotype in the < 65 years group [2.797 (0.843 to 9.280) as compared to those > 65 years was 0.838 (0.298 to 2.357)]. These sub-group results suggest there might be a higher degree of DNA damage in younger individuals with the variant allele. This merits further investigation. It has recently been demonstrated that older (more than 47 yrs) healthy Japanese workers with the XRCC1 Arg/Arg allele have a higher degree of DNA damage as measured by the comet assay(Weng et al., 2009), but our results are at odds with these findings. We however should entertain the idea that our result could be secondary to the design of our study rather than a genuine finding.

XRCC1 Arg280His

XRCC1 280 genotype did not confer a significant risk for pancreatic cancer.

Our study has been the first to explore XRCC1 Arg280His and risk for pancreatic cancer. However risk for other tobacco related cancers eg lung cancer has been assessed for the genotype and XRCC1 Arg280His did not reveal either an overall effect or an interaction between genotype, tobacco smoking and risk for lung cancer (Schneider et al., 2005). Large-scale investigation of the risk for XRCC1 genes (including XRCC1 Arg280his) has not revealed a significant role for these SNP's in lung cancer risk (Hung et al., 2005b).

Recent meta-analysis of studies exploring the role of XRCC1 (Arg194Trp, Arg280His and Arg399Gln) have concluded that there is no increased risk for either gastric (Xue et al., 2011) or colorectal (Wang et al., 2010) cancer associated with the genotypes. However one other meta-analysis suggested that the variant XRCC1 Arg194Trp allele was associated with an increased risk for gastric cancer, while agreeing hat there was no increased risk associated with the other two widely studied XRCC1 genotypes (Chen et al., 2011).

APE1Asp148Gln

APE1 Asp148Gln did not demonstrate a significant risk for pancreas cancer.
A previous study investigating this SNP in 384 cases and 357 controls has not found a main association or interaction between Ape1 Asp148Gln and tobacco smoking (Jiao et al., 2006b).

Functional characterization of APE1 variants has not revealed significant chromosomal alterations associated with this SNP (Au et al., 2003), also no specific had no impact on endonuclease and DNA binding activities (Hadi et al., 2000).

A recent study has explored the potential prognostic role of APE1 expression on pancreato-biliary cancers (Al-Attar et al., 2010) (pancreas adenocarcinoma, n=34) by immune-histochemical staining of formalin-fixed tissue. In pancreatico-biliary cancer, nuclear staining was seen in 44% (32 out of 72) of tumours. Absence of cytoplasmic staining was associated with perineural invasion (p=0.007), vascular invasion (p=0.05), and poorly differentiated tumours (p=0.068). A trend was noticed with advanced stage disease (p=0.077).

5.3: Summary and Further investigation

The present study has some strengths. For example we have minimized the outcome misclassification by attempting to obtain a histological diagnosis to a large extent, with 122 of our 164 cases pathologically confirmed (74.3%). Performing direct interviews for collecting exposure information reduced information obtainment bias and one single individual did all these interviews.

Our study has important limitations. Voluntary participation of the patients and controls might have introduced bias to the association investigation. However, it seems unlikely that willingness to participate in research would affect genotype frequencies for DNA repair enzyme genes. Also our study has a mixture of current (those who attended hospital for illnesses not related to the pancreas) and historical controls from another research study. Importantly the possibility of chance findings cannot be excluded because of the small sample size and the low allele frequencies. The current study is underpowered in examining the gene-environment interaction. Alcohol consumption, which is a confounding factor, has not been factored into this study, as we have not obtained alcohol consumption information from our study participants. Another major deficiency in the current study is the incomplete family history data collection – specifically we did not collect the total number of FDR's in a specific family and also the age of onset of malignancy in them (if any) and their current health status. If this had been collected an attempt to assess standardized risk ratios could have been made. Due to all these reasons, the interpretation of our findings needs caution.

In our cohort of patients with pancreatic cancer, the total tobacco exposure was significantly lower amongst cases with a history of malignancy in FDR compared to cases who were smokers who did not have such a history. This sub-group of patients

should be a focus for further investigation to advance understanding gene-environment interaction in this disease.

Investigation of this gene-environment interaction provides us with an opportunity to not only understand the disease better but also to stratify risks and develop strategies to improve outcome. This inter-individual genetic variation modulates risk for malignancy (Lochan et al., 2008) and identification of these genetic differences forms the basis of risk stratification thereby enabling targeted prevention or earlier diagnosis (Singh and Maitra, 2007, Vimalachandran et al., 2004). This is especially pertinent to pancreatic cancer, as it has a particularly poor prognosis and palliation of symptoms is the most common therapy patients receive – mainly because of late diagnosis although there are other biological factors that play a role. Genetic factors such as poor DNA repair, impaired carcinogen metabolism and environmental factors may interact in the development of tobacco related cancers, including that of the lung, bladder and head and neck (Greer and Whitcomb, 2007) (Wiencke, 2002) (Hung et al., 2005c, Barnes and Lindahl, 2004).

As discussed earlier (Section 1.6) conflicting results have emerged from studies on XRCC1 and other gene polymorphisms and cancer risks. Studies have been hampered by small sample sizes and study design. Another important challenge is that the measurement of environmental influences particularly, tobacco exposure, needs to be improved to better define gene–environment interaction. None of these studies, however, has ascertained the risk for smokers carrying these genotypes in the presence of a family history of malignancy.

Sequence variants in DNA repair genes are thought to modulate DNA repair capacity and consequently are suggested to be associated with altered cancer risk. However, results from epidemiologic studies have been inconsistent and relatively small risks

have been identified (including our own), possibly because of 1) low statistical power for detecting a moderate effect, 2) false-positive results 3) heterogeneity across study populations, 4) failure to consider effect modifiers such as environmental exposures, and 5) publication bias. Reliable knowledge of which sequence variants influence cancer risk may help in identifying persons at high risk of developing cancer and shed light on cancer aetiology.

The presence of a high-risk group for adenocarcinoma of the pancreas is well accepted, which needs further characterization and replication in much larger population based and molecular epidemiological studies. Identifying risk might help stratify individuals for pancreatic cancer screening but screening is not well established, the pickup rate is low and the false positive rate is relatively high. This will require identification of high-risk groups in whom targeted screening can be employed and early/precursor lesions recognized (Klapman and Malafa, 2008) and this has been demonstrated successfully in familial forms of the disease (Canto et al., 2004) and has been found to be cost-effective (Rulyak et al., 2003b). A recent retrospective review comparing EUS and ERCP has demonstrated the superiority of EUS (Wakatsuki et al., 2005) for the diagnosis of pancreatic masses. EUS of the pancreas has been utilised as a screening tool(Rubenstein et al., 2007), where it was used as part of 4 strategies: doing nothing, prophylactic total pancreatectomy, annual surveillance by EUS, and annual surveillance with EUS and fine needle aspiration (EUS/FNA). FDR's from a familial cancer kindred were subjected to one of the 4 strategies and the authors concluded that FDRs from familial pancreatic cancer kindreds, who have EUS findings of chronic pancreatitis, have increased risk for cancer, but their precise risk was unknown. Without the ability to further quantify that risk, the most effective strategy is to do nothing. However if we are able to better quantify the risk, the benefits might be greater for these patients as surgery usually means a total pancreatectomy with all its potential complications.

Identifying genetic and environmental factors and delineating their complex interaction is important in this regard.

Similarly identification of high-risk groups such as smokers with a positive family history of cancer could have implications for the earlier diagnosis by making screening for the disease possible leading to the prospect of long-term survival, if not cure for more patients. Following significant advances in imaging to aid in patient selection for definitive treatment and improvement in surgical technique and peri-operative care, prognosis for resectable pancreatic cancer has improved appreciably. Chemotherapy has a significant role to play in selected cases (Aung et al., 2007). However it does appear that further significant improvement in outcome from the illness will be directly related to the ability to detect the disease early and institute prompt management. This will require identification of high-risk groups in whom targeted screening can be employed and early or precursor lesions recognized (Klapman and Malafa, 2008) and this has been demonstrated successfully in familial forms of the disease (Canto et al., 2004) and has been found to be cost-effective (Rulyak et al., 2003b).

Also there is increasing evidence for the modulation of outcome from pancreatic cancer by genotype for DNA repair mechanisms (Li et al., 2007b, Li et al., 2006c) including response to chemotherapy and pre-operative chemo-radiation (Dong et al., 2009) (Okazaki et al., 2008). Defining these risks and benefits might allow selection of patients for specific neo-adjuvant and adjuvant treatment strategies.

With the completion of the human genome project and advances in molecular epidemiological techniques, these low penetrant/polymorphic genes should become more frequently identified and their function understood; for example genome wide association studies (GWAS) have identified smokers with a non- O blood group as a significant high risk group for pancreas cancer as compared to non-smokers of non-O

blood group [OR 2.68 (95% CI, 2.03-3.54)] (Amundadottir et al.). The same group has carried out a further GWAS (Petersen et al., 2010) and have reported the association for pancreatic cancer and three new genomic regions on chromosomes 13q22.1 (rs9543325 and rs9564966), 1q32.1 (5 highly significant SNPs map to this region) and 5p15.33 (rs401681). The area identified on chromosome 1 contains the nuclear receptor subfamily 5, group A, member 2 (NR5A2) gene which plays a role in early development of embryos. The region identified on chromosome 5 has been identified in genome-wide association studies of a number of different cancers, including brain tumors, lung cancer, basal cell carcinoma, melanoma. Furthermore in an analysis of lung cancer in smokers, the signal on chromosome 5p15.33 has been shown to be strongly associated with the adenocarcinoma histology subtype. Also, SNP's in this region, have been associated with levels of smoking-related bulky aromatic DNA adducts in lung cancer patients (Zienolddiny et al., 2006) and tobacco smoking is a known and relevant risk factor for pancreatic cancer. The area identified on chromosome 13 is frequently deleted in a range of cancers, including pancreatic cancer and may harbor a breast cancer susceptibility locus. In a GWAS from Japan three genomic regions, 6p25.3, 12p11.21 and 7q36.2, were shown to be significantly associated with increased risk of pancreatic cancer (Low et al., 2010) and rs9502893 the most significantly associated SNP is located within the FOXq1 gene complex. A member of this gene FOXM1 is overexpressed in pancreatic cancer. Another SNP from this GWAS, rs708224 was significantly associated with the disease and it is located within the BICD1 gene, which is linked to vacuolar trafficking and telomere function, and there has been some recent evidence to link this to pancreatic cancer.

Thus, the elusive search for a better understanding of this disease continues and it is undoubtedly the case that a better outcome from pancreatic cancer will result from a better understanding of the genetics and epidemiology of the disease.

REFERENCES

2004. The Health Consequences of Smoking: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services. Department of Health and Human

Services, Centers for Disease Control and Prevention, National Center for Chronic

Disease Prevention and Health Promotion, Office on Smoking and Health.

ABDEL-RAHMAN, S. Z. & EL-ZEIN, R. A. 2000. The 399Gln polymorphism in the DNA repair gene XRCC1 modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. *Cancer Lett,* 159**,** 63-71.

AHLGREN, J. D. 1996. Epidemiology and risk factors in pancreatic cancer. *Semin Oncol,* 23**,** 241-50.

AL-ATTAR, A., GOSSAGE, L., FAREED, K. R., SHEHATA, M., MOHAMMED, M., ZAITOUN, A. M., SOOMRO, I., LOBO, D. N., ABBOTTS, R., CHAN, S. & MADHUSUDAN, S. 2010. Human apurinic/apyrimidinic endonuclease (APE1) is a prognostic factor in ovarian, gastro-oesophageal and pancreatico-biliary cancers. *British journal of cancer,* 102**,** 704-9.

AMUNDADOTTIR, L., KRAFT, P., STOLZENBERG-SOLOMON, R. Z., FUCHS, C. S., PETERSEN, G. M., ARSLAN, A. A., BUENO-DE-MESQUITA, H. B., GROSS, M., HELZLSOUER, K., JACOBS, E. J., LACROIX, A., ZHENG, W., ALBANES, D., BAMLET, W., BERG, C. D., BERRINO, F., BINGHAM, S., BURING, J. E., BRACCI, P. M., CANZIAN, F., CLAVEL-CHAPELON, F., CLIPP, S., COTTERCHIO, M., DE ANDRADE, M., DUELL, E. J., FOX, J. W., JR., GALLINGER, S., GAZIANO, J. M., GIOVANNUCCI, E. L., GOGGINS, M., GONZALEZ, C. A., HALLMANS, G., HANKINSON, S. E., HASSAN, M., HOLLY, E. A., HUNTER, D. J., HUTCHINSON, A., JACKSON, R., JACOBS, K. B., JENAB, M., KAAKS, R., KLEIN, A. P., KOOPERBERG, C., KURTZ, R. C., LI, D., LYNCH, S. M., MANDELSON, M., MCWILLIAMS, R. R., MENDELSOHN, J. B., MICHAUD, D. S., OLSON, S. H., OVERVAD, K., PATEL, A. V., PEETERS, P. H., RAJKOVIC, A., RIBOLI, E., RISCH, H. A., SHU, X. O., THOMAS, G., TOBIAS, G. S., TRICHOPOULOS, D., VAN DEN EEDEN, S. K., VIRTAMO, J., WACTAWSKI-WENDE, J., WOLPIN, B. M., YU, H., YU, K., ZELENIUCH-JACQUOTTE, A., CHANOCK, S. J., HARTGE, P. & HOOVER, R. N. 2009. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat Genet,* 41**,** 986-90.

ANDERSON, K. E., HAMMONS, G. J., KADLUBAR, F. F., POTTER, J. D., KADERLIK, K. R., ILETT, K. F., MINCHIN, R. F., TEITEL, C. H., CHOU, H. C., MARTIN, M. V., GUENGERICH, F. P., BARONE, G. W., LANG, N. P. & PETERSON, L. A. 1997. Metabolic activation of aromatic amines by human pancreas. *Carcinogenesis,* 18**,** 1085-92.

ANDRE, T., BALOSSO, J., LOUVET, C., GLIGOROV, J., CALLARD, P., DE GRAMONT, A. & IZRAEL, V. 1998. [Adenocarcinoma of the pancreas. General characteristics]. *Presse Med,* 27**,** 533-6.

AOKI, K. & OGAWA, H. 1978. Cancer of the pancreas, international mortality trends. *World Health Stat Q,* 31**,** 2-27.

AQUILINA, G. & BIGNAMI, M. 2001. Mismatch repair in correction of replication errors and processing of DNA damage. *J Cell Physiol,* 187**,** 145-54.

AU, W. W., SALAMA, S. A. & SIERRA-TORRES, C. H. 2003. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. *Environmental health perspectives,* 111**,** 1843-50.

AUNG, K. L., SMITH, D. B. & NEOPTOLEMOS, J. P. 2007. Adjuvant therapy for pancreatic cancer. *Expert Opin Pharmacother,* 8**,** 2533-41.

BAGHURST, P. A., MCMICHAEL, A. J., SLAVOTINEK, A. H., BAGHURST, K. I., BOYLE, P. & WALKER, A. M. 1991. A case-control study of diet and cancer of the pancreas. *Am J Epidemiol,* 134**,** 167-79.

BARNES, D. E. & LINDAHL, T. 2004. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet,* 38**,** 445-76.

BARTSCH, H. & HIETANEN, E. 1996. The role of individual susceptibility in cancer burden related to environmental exposure. *Environmental health perspectives,* 104 Suppl 3**,** 569-77.

BEGGS, A. D., LATCHFORD, A. R., VASEN, H. F., MOSLEIN, G., ALONSO, A., ARETZ, S., BERTARIO, L., BLANCO, I., BULOW, S., BURN, J., CAPELLA, G., COLAS, C., FRIEDL, W., MOLLER, P., HES, F. J., JARVINEN, H., MECKLIN, J. P., NAGENGAST, F. M., PARC, Y., PHILLIPS, R. K., HYER, W., PONZ DE LEON, M., RENKONEN-SINISALO, L., SAMPSON, J. R., STORMORKEN, A., TEJPAR, S., THOMAS, H. J., WIJNEN, J. T., CLARK, S. K. & HODGSON, S. V. 2010. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut,* 59**,** 975-86.

BEN, Q., XU, M., NING, X., LIU, J., HONG, S., HUANG, W., ZHANG, H. & LI, Z. 2011. Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. *European journal of cancer,* 47**,** 1928-37.

BERGER, A. C., GARCIA, M., JR., HOFFMAN, J. P., REGINE, W. F., ABRAMS, R. A., SAFRAN, H., KONSKI, A., BENSON, A. B., 3RD, MACDONALD, J. & WILLETT, C. G. 2008. Postresection CA 19-9 predicts overall survival in patients with pancreatic cancer treated with adjuvant chemoradiation: a prospective validation by RTOG 9704. *J Clin Oncol,* 26**,** 5918-22.

BESARATINIA, A., VAN SCHOOTEN, F. J., SCHILDERMAN, P. A., DE KOK, T. M., HAENEN, G. R., VAN HERWIJNEN, M. H., VAN AGEN, E., PACHEN, D. & KLEINJANS, J. C. 2001. A multi-biomarker approach to study the effects of smoking on oxidative DNA damage and repair and antioxidative defense mechanisms. *Carcinogenesis,* 22**,** 395-401.

BOHR, V. A. & ANSON, R. M. 1995. DNA damage, mutation and fine structure DNA repair in aging. *Mutat Res,* 338**,** 25-34.

BOLAND, C. R., THIBODEAU, S. N., HAMILTON, S. R., SIDRANSKY, D., ESHLEMAN, J. R., BURT, R. W., MELTZER, S. J., RODRIGUEZ-BIGAS, M. A., FODDE, R., RANZANI, G. N. & SRIVASTAVA, S. 1998. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res,* 58**,** 5248-57.

BOWLING, A. 2005. Mode of questionnaire administration can have serious effects on data quality. *Journal of public health,* 27**,** 281-91.

BOYLE, P., HSIEH, C. C., MAISONNEUVE, P., LA VECCHIA, C., MACFARLANE, G. J., WALKER, A. M. & TRICHOPOULOS, D. 1989. Epidemiology of pancreas cancer (1988). *Int J Pancreatol,* 5**,** 327-46.

BOYLE, P., MAISONNEUVE, P., BUENO DE MESQUITA, B., GHADIRIAN, P., HOWE, G. R., ZATONSKI, W., BAGHURST, P., MOERMAN, C. J., SIMARD, A., MILLER, A. B., PRZEWONIAK, K., MCMICHAEL, A. J., HSIEH, C. C. & WALKER, A. M. 1996. Cigarette smoking and pancreas cancer: a case control study of the search programme of the IARC. *Int J Cancer,* 67**,** 63-71.

BRACCI, P. M. 2012. Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. *Molecular carcinogenesis,* 51**,** 53-63.
BRENTNALL, T. A. 2005. Management strategies for patients with hereditary pancreatic cancer. *Curr Treat Options Oncol,* 6**,** 437-45.
BRUGGE, W. R., LEWANDROWSKI, K., LEE-LEWANDROWSKI, E., CENTENO, B. A., SZYDLO, T., REGAN, S., DEL CASTILLO, C. F. & WARSHAW, A. L. 2004. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology,* 126**,** 1330-6.
BUENO DE MESQUITA, H. B., MAISONNEUVE, P., MOERMAN, C. J., RUNIA, S. & BOYLE, P. 1991. Life-time history of smoking and exocrine carcinoma of the pancreas: a population-based case-control study in The Netherlands. *Int J Cancer,* 49**,** 816-22.
BURRIS, H. A., 3RD, MOORE, M. J., ANDERSEN, J., GREEN, M. R., ROTHENBERG, M. L., MODIANO, M. R., CRIPPS, M. C., PORTENOY, R. K., STORNIOLO, A. M., TARASSOFF, P., NELSON, R., DORR, F. A., STEPHENS, C. D. & VON HOFF, D. D. 1997. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol,* 15**,** 2403-13.
CALDAS, C., HAHN, S. A., HRUBAN, R. H., REDSTON, M. S., YEO, C. J. & KERN, S. E. 1994. Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. *Cancer Res,* 54**,** 3568-73.
CALDECOTT, K. W. 2003. XRCC1 and DNA strand break repair. *DNA Repair (Amst),* 2**,** 955-69.
CANTO, M. I. 2007. Strategies for screening for pancreatic adenocarcinoma in high-risk patients. *Semin Oncol,* 34**,** 295-302.
CANTO, M. I., GOGGINS, M., HRUBAN, R. H., PETERSEN, G. M., GIARDIELLO, F. M., YEO, C., FISHMAN, E. K., BRUNE, K., AXILBUND, J., GRIFFIN, C., ALI, S., RICHMAN, J., JAGANNATH, S., KANTSEVOY, S. V. & KALLOO, A. N. 2006. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clin Gastroenterol Hepatol,* 4**,** 766-81; quiz 665.
CANTO, M. I., GOGGINS, M., YEO, C. J., GRIFFIN, C., AXILBUND, J. E., BRUNE, K., ALI, S. Z., JAGANNATH, S., PETERSEN, G. M., FISHMAN, E. K., PIANTADOSI, S., GIARDIELLO, F. M. & HRUBAN, R. H. 2004. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. *Clin Gastroenterol Hepatol,* 2**,** 606-21.
CARIUK, P., LORITE, M. J., TODOROV, P. T., FIELD, W. N., WIGMORE, S. J. & TISDALE, M. J. 1997. Induction of cachexia in mice by a product isolated from the urine of cachectic cancer patients. *British journal of cancer,* 76**,** 606-13.
CHEN, B., ZHOU, Y., YANG, P. & WU, X. T. 2011. Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis. *Molecular biology reports*.
CHEN, L., AMBROSONE, C. B., LEE, J., SELLERS, T. A., POW-SANG, J. & PARK, J. Y. 2006. Association between polymorphisms in the DNA repair genes XRCC1 and APE1, and the risk of prostate cancer in white and black Americans. *J Urol,* 175**,** 108-12; discussion 112.
CHO, E. Y., HILDESHEIM, A., CHEN, C. J., HSU, M. M., CHEN, I. H., MITTL, B. F., LEVINE, P. H., LIU, M. Y., CHEN, J. Y., BRINTON, L. A., CHENG, Y. J. & YANG, C. S. 2003. Nasopharyngeal carcinoma and genetic polymorphisms of DNA repair enzymes XRCC1 and hOGG1. *Cancer Epidemiol Biomarkers Prev,* 12**,** 1100-4.
CHOI, B. C. & PAK, A. W. 2005. A catalog of biases in questionnaires. *Preventing chronic disease,* 2**,** A13.

CHOI, J. Y., HAMAJIMA, N., TAJIMA, K., YOO, K. Y., YOON, K. S., PARK, S. K., KIM, S. U., LEE, K. M., NOH, D. Y., AHN, S. H., CHOE, K. J., HAN, W., HIRVONEN, A. & KANG, D. 2003. hOGG1 Ser326Cys polymorphism and breast cancer risk among Asian women. *Breast Cancer Res Treat,* 79**,** 59-62.

CHUA, T. C. & SAXENA, A. 2010. Extended pancreaticoduodenectomy with vascular resection for pancreatic cancer: a systematic review. *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract,* 14**,** 1442-52.

CILIBERTO, D., BOTTA, C., CORREALE, P., ROSSI, M., CARAGLIA, M., TASSONE, P. & TAGLIAFERRI, P. 2013. Role of gemcitabine-based combination therapy in the management of advanced pancreatic cancer: a meta-analysis of randomised trials. *European journal of cancer,* 49**,** 593-603.

COLAS, C., COULET, F., SVRCEK, M., COLLURA, A., FLEJOU, J. F., DUVAL, A. & HAMELIN, R. 2012. Lynch or not Lynch? Is that always a question? *Advances in cancer research,* 113**,** 121-66.

COLLINS, A. R. 1998. Molecular epidemiology in cancer research. *Molecular aspects of medicine,* 19**,** 359-432.

CONLON, K. C., KLIMSTRA, D. S. & BRENNAN, M. F. 1996. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. *Ann Surg,* 223**,** 273-9.

CONROY, T., DESSEIGNE, F., YCHOU, M., BOUCHE, O., GUIMBAUD, R., BECOUARN, Y., ADENIS, A., RAOUL, J. L., GOURGOU-BOURGADE, S., DE LA FOUCHARDIERE, C., BENNOUNA, J., BACHET, J. B., KHEMISSA-AKOUZ, F., PERE-VERGE, D., DELBALDO, C., ASSENAT, E., CHAUFFERT, B., MICHEL, P., MONTOTO-GRILLOT, C. & DUCREUX, M. 2011. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *The New England journal of medicine,* 364**,** 1817-25.

COUCH, F. J., JOHNSON, M. R., RABE, K. G., BRUNE, K., DE ANDRADE, M., GOGGINS, M., ROTHENMUND, H., GALLINGER, S., KLEIN, A., PETERSEN, G. M. & HRUBAN, R. H. 2007. The prevalence of BRCA2 mutations in familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev,* 16**,** 342-6.

CUNNINGHAM, D., CHAU, I., STOCKEN, D. D., VALLE, J. W., SMITH, D., STEWARD, W., HARPER, P. G., DUNN, J., TUDUR-SMITH, C., WEST, J., FALK, S., CRELLIN, A., ADAB, F., THOMPSON, J., LEONARD, P., OSTROWSKI, J., EATOCK, M., SCHEITHAUER, W., HERRMANN, R. & NEOPTOLEMOS, J. P. 2009a. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol,* 27**,** 5513-8.

CUNNINGHAM, D., CHAU, I., STOCKEN, D. D., VALLE, J. W., SMITH, D., STEWARD, W., HARPER, P. G., DUNN, J., TUDUR-SMITH, C., WEST, J., FALK, S., CRELLIN, A., ADAB, F., THOMPSON, J., LEONARD, P., OSTROWSKI, J., EATOCK, M., SCHEITHAUER, W., HERRMANN, R. & NEOPTOLEMOS, J. P. 2009b. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* 27**,** 5513-8.

CUZICK, J., ROUTLEDGE, M. N., JENKINS, D. & GARNER, R. C. 1990. DNA adducts in different tissues of smokers and non-smokers. *Int J Cancer,* 45**,** 673-8.

DALY, A. K., KING, B. P. & LEATHART, J. B. 2006. Genotyping for cytochrome P450 polymorphisms. *Methods in molecular biology,* 320**,** 193-207.

DAVID, S. S., O'SHEA, V. L. & KUNDU, S. 2007. Base-excision repair of oxidative DNA damage. *Nature,* 447**,** 941-50.

DAY, J. D., DIGIUSEPPE, J. A., YEO, C., LAI-GOLDMAN, M., ANDERSON, S. M., GOODMAN, S. N., KERN, S. E. & HRUBAN, R. H. 1996. Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. *Hum Pathol,* 27**,** 119-24.

DEL CHIARO, M., ZERBI, A., FALCONI, M., BERTACCA, L., POLESE, M., SARTORI, N., BOGGI, U., CASARI, G., LONGONI, B. M., SALVIA, R., CALIGO, M. A., DI CARLO, V., PEDERZOLI, P., PRESCIUTTINI, S. & MOSCA, F. 2007. Cancer risk among the relatives of patients with pancreatic ductal adenocarcinoma. *Pancreatology,* 7**,** 459-69.

DHENAUT, A., BOITEUX, S. & RADICELLA, J. P. 2000. Characterization of the hOGG1 promoter and its expression during the cell cycle. *Mutat Res,* 461**,** 109-18.

DIANOV, G. L., SLEETH, K. M., DIANOVA, II & ALLINSON, S. L. 2003. Repair of abasic sites in DNA. *Mutat Res,* 531**,** 157-63.

DIGIUSEPPE, J. A., HRUBAN, R. H., GOODMAN, S. N., POLAK, M., VAN DEN BERG, F. M., ALLISON, D. C., CAMERON, J. L. & OFFERHAUS, G. J. 1994. Overexpression of p53 protein in adenocarcinoma of the pancreas. *Am J Clin Pathol,* 101**,** 684-8.

DING, Y., CRAVERO, J. D., ADRIAN, K. & GRIPPO, P. 2010. Modeling pancreatic cancer in vivo: from xenograft and carcinogen-induced systems to genetically engineered mice. *Pancreas,* 39**,** 283-92.

DOLL, R., PETO, R., WHEATLEY, K., GRAY, R. & SUTHERLAND, I. 1994. Mortality in relation to smoking: 40 years' observations on male British doctors. *Bmj,* 309**,** 901-11.

DONG, X., JIAO, L., LI, Y., EVANS, D. B., WANG, H., HESS, K. R., ABBRUZZESE, J. L. & LI, D. 2009. Significant associations of mismatch repair gene polymorphisms with clinical outcome of pancreatic cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology,* 27**,** 1592-9.

DUELL, E. J. 2012. Epidemiology and potential mechanisms of tobacco smoking and heavy alcohol consumption in pancreatic cancer. *Molecular carcinogenesis,* 51**,** 40-52.

DUELL, E. J., HOLLY, E. A., BRACCI, P. M., LIU, M., WIENCKE, J. K. & KELSEY, K. T. 2002a. A population-based, case-control study of polymorphisms in carcinogen-metabolizing genes, smoking, and pancreatic adenocarcinoma risk. *J Natl Cancer Inst,* 94**,** 297-306.

DUELL, E. J., HOLLY, E. A., BRACCI, P. M., WIENCKE, J. K. & KELSEY, K. T. 2002b. A population-based study of the Arg399Gln polymorphism in X-ray repair cross- complementing group 1 (XRCC1) and risk of pancreatic adenocarcinoma. *Cancer Res,* 62**,** 4630-6.

DUMONCEAU, J. M., TRINGALI, A., BLERO, D., DEVIERE, J., LAUGIERS, R., HERESBACH, D. & COSTAMAGNA, G. 2012. Biliary stenting: indications, choice of stents and results: European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. *Endoscopy,* 44**,** 277-98.

EARL, J., YAN, L., VITONE, L. J., RISK, J., KEMP, S. J., MCFAUL, C., NEOPTOLEMOS, J. P., GREENHALF, W., KRESS, R., SINA-FREY, M., HAHN, S. A., RIEDER, H. & BARTSCH, D. K. 2006. Evaluation of the 4q32-34 locus in European familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev,* 15**,** 1948-55.

EBERLE, M. A., PFUTZER, R., POGUE-GEILE, K. L., BRONNER, M. P., CRISPIN, D., KIMMEY, M. B., DUERR, R. H., KRUGLYAK, L., WHITCOMB, D. C. & BRENTNALL, T. A. 2002. A new susceptibility locus for autosomal dominant pancreatic cancer maps to chromosome 4q32-34. *Am J Hum Genet,* 70**,** 1044-8.

ELAHI, A., ZHENG, Z., PARK, J., EYRING, K., MCCAFFREY, T. & LAZARUS, P. 2002. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. *Carcinogenesis,* 23**,** 1229-34.

ENGELAND, A., ANDERSEN, A., HALDORSEN, T. & TRETLI, S. 1996. Smoking habits and risk of cancers other than lung cancer: 28 years' follow-up of 26,000 Norwegian men and women. *Cancer Causes Control,* 7**,** 497-506.

EPSTEIN, A. S. & O'REILLY, E. M. 2012. Exocrine pancreas cancer and thromboembolic events: a systematic literature review. *Journal of the National Comprehensive Cancer Network : JNCCN,* 10**,** 835-46.

ESHUIS, W. J., VAN DER GAAG, N. A., RAUWS, E. A., VAN EIJCK, C. H., BRUNO, M. J., KUIPERS, E. J., COENE, P. P., KUBBEN, F. J., GERRITSEN, J. J., GREVE, J. W., GERHARDS, M. F., DE HINGH, I. H., KLINKENBIJL, J. H., NIO, C. Y., DE CASTRO, S. M., BUSCH, O. R., VAN GULIK, T. M., BOSSUYT, P. M. & GOUMA, D. J. 2010. Therapeutic delay and survival after surgery for cancer of the pancreatic head with or without preoperative biliary drainage. *Ann Surg,* 252**,** 840-9.

EVANS, D. B., VARADHACHARY, G. R., CRANE, C. H., SUN, C. C., LEE, J. E., PISTERS, P. W., VAUTHEY, J. N., WANG, H., CLEARY, K. R., STAERKEL, G. A., CHARNSANGAVEJ, C., LANO, E. A., HO, L., LENZI, R., ABBRUZZESE, J. L. & WOLFF, R. A. 2008. Preoperative gemcitabine-based chemoradiation for patients with resectable adenocarcinoma of the pancreatic head. *J Clin Oncol,* 26**,** 3496-502.

FAN, J., OTTERLEI, M., WONG, H. K., TOMKINSON, A. E. & WILSON, D. M., 3RD 2004. XRCC1 co-localizes and physically interacts with PCNA. *Nucleic Acids Res,* 32**,** 2193-201.

FENDRICH, V. & BARTSCH, D. K. 2005. [Hereditary gastrointestinal neoplasias]. *Z Gastroenterol,* 43**,** 219-25.

FENG, Z., HU, W., CHEN, J. X., PAO, A., LI, H., ROM, W., HUNG, M. C. & TANG, M. S. 2002. Preferential DNA damage and poor repair determine ras gene mutational hotspot in human cancer. *J Natl Cancer Inst,* 94**,** 1527-36.

FINCH, M. D., HOWES, N., ELLIS, I., MOUNTFORD, R., SUTTON, R., RARATY, M. & NEOPTOLEMOS, J. P. 1997. Hereditary pancreatitis and familial pancreatic cancer. *Digestion,* 58**,** 564-9.

FISCHER, L., FRIESS, H., Z'GRAGGEN, K., UHL, W. & BUCHLER, M. W. 2003. [Operative management in the treatment of pancreatic cancer]. *Zentralbl Chir,* 128**,** 390-5.

FLANDERS, T. Y. & FOULKES, W. D. 1996. Pancreatic adenocarcinoma: epidemiology and genetics. *J Med Genet,* 33**,** 889-98.

FRAUMENI, J. F., JR. 1975. Cancers of the pancreas and biliary tract: epidemiological considerations. *Cancer Res,* 35**,** 3437-46.

FRENCH, J. J., MANSFIELD, S. D., JAQUES, K., JAQUES, B. C., MANAS, D. M. & CHARNLEY, R. M. 2009. Fast-track management of patients undergoing proximal pancreatic resection. *Annals of the Royal College of Surgeons of England,* 91**,** 201-4.

FUCHS, C. S., COLDITZ, G. A., STAMPFER, M. J., GIOVANNUCCI, E. L., HUNTER, D. J., RIMM, E. B., WILLETT, W. C. & SPEIZER, F. E. 1996. A prospective study of cigarette smoking and the risk of pancreatic cancer. *Arch Intern Med,* 156**,** 2255-60.

FUJINO, Y., MATSUMOTO, I., UEDA, T., TOYAMA, H. & KURODA, Y. 2007. Proposed new score predicting malignancy of intraductal papillary mucinous neoplasms of the pancreas. *Am J Surg,* 194**,** 304-7.

FUKUKURA, Y., FUJIYOSHI, F., HAMADA, H., TAKAO, S., AIKOU, T., HAMADA, N., YONEZAWA, S. & NAKAJO, M. 2003. Intraductal papillary mucinous tumors of the pancreas. Comparison of helical CT and MR imaging. *Acta Radiol,* 44**,** 464-71.

GACKOWSKI, D., SPEINA, E., ZIELINSKA, M., KOWALEWSKI, J., ROZALSKI, R., SIOMEK, A., PACIOREK, T., TUDEK, B. & OLINSKI, R. 2003. Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. *Cancer Res,* 63**,** 4899-902.

GARCEA, G., DENNISON, A. R., PATTENDEN, C. J., NEAL, C. P., SUTTON, C. D. & BERRY, D. P. 2008a. Survival following curative resection for pancreatic ductal adenocarcinoma. A systematic review of the literature. *JOP,* 9**,** 99-132.

GARCEA, G., ONG, S. L., RAJESH, A., NEAL, C. P., POLLARD, C. A., BERRY, D. P. & DENNISON, A. R. 2008b. Cystic lesions of the pancreas. A diagnostic and management dilemma. *Pancreatology,* 8**,** 236-51.

GARDNER, J. W. & LYON, J. L. 1982. Cancer in Utah Mormon men by lay priesthood level. *Am J Epidemiol,* 116**,** 243-57.

GARFINKEL, L. 1991. The epidemiology of cancer in black Americans. *Stat Bull Metrop Insur Co,* 72**,** 11-7.

GENKINGER, J. M., SPIEGELMAN, D., ANDERSON, K. E., BERGKVIST, L., BERNSTEIN, L., VAN DEN BRANDT, P. A., ENGLISH, D. R., FREUDENHEIM, J. L., FUCHS, C. S., GILES, G. G., GIOVANNUCCI, E., HANKINSON, S. E., HORN-ROSS, P. L., LEITZMANN, M., MANNISTO, S., MARSHALL, J. R., MCCULLOUGH, M. L., MILLER, A. B., REDING, D. J., ROBIEN, K., ROHAN, T. E., SCHATZKIN, A., STEVENS, V. L., STOLZENBERG-SOLOMON, R. Z., VERHAGE, B. A., WOLK, A., ZIEGLER, R. G. & SMITH-WARNER, S. A. 2009. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology,* 18**,** 765-76.

GHADIRIAN, P., BOYLE, P., SIMARD, A., BAILLARGEON, J., MAISONNEUVE, P. & PERRET, C. 1991. Reported family aggregation of pancreatic cancer within a population-based case-control study in the Francophone community in Montreal, Canada. *Int J Pancreatol,* 10**,** 183-96.

GHADIRIAN, P., LIU, G., GALLINGER, S., SCHMOCKER, B., PARADIS, A. J., LAL, G., BRUNET, J. S., FOULKES, W. D. & NAROD, S. A. 2002. Risk of pancreatic cancer among individuals with a family history of cancer of the pancreas. *Int J Cancer,* 97**,** 807-10.

GHIMENTI, C., TANNERGARD, P., WAHLBERG, S., LIU, T., GIULIANOTTI, P. G., MOSCA, F., FORNACIARI, G., BEVILACQUA, G., LINDBLOM, A. & CALIGO, M. A. 1999. Microsatellite instability and mismatch repair gene inactivation in sporadic pancreatic and colon tumours. *British journal of cancer,* 80**,** 11-6.

GIARDIELLO, F. M., BRENSINGER, J. D., TERSMETTE, A. C., GOODMAN, S. N., PETERSEN, G. M., BOOKER, S. V., CRUZ-CORREA, M. & OFFERHAUS, J. A. 2000. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology,* 119**,** 1447-53.

GIARDIELLO, F. M., OFFERHAUS, G. J., LEE, D. H., KRUSH, A. J., TERSMETTE, A. C., BOOKER, S. V., KELLEY, N. C. & HAMILTON, S. R. 1993. Increased

risk of thyroid and pancreatic carcinoma in familial adenomatous polyposis. *Gut,* 34**,** 1394-6.
GOGGINS, M., HRUBAN, R. H. & KERN, S. E. 2000. BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: evidence and implications. *Am J Pathol,* 156**,** 1767-71.
GOGGINS, M., SCHUTTE, M., LU, J., MOSKALUK, C. A., WEINSTEIN, C. L., PETERSEN, G. M., YEO, C. J., JACKSON, C. E., LYNCH, H. T., HRUBAN, R. H. & KERN, S. E. 1996. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res,* 56**,** 5360-4.
GOLD, E. B. & GOLDIN, S. B. 1998. Epidemiology of and risk factors for pancreatic cancer. *Surg Oncol Clin N Am,* 7**,** 67-91.
GREENBERG, M. & SCHNEIDER, D. 1995. The cancer burden of southern-born African Americans: analysis of social-geographic legacy. *Milbank Q,* 73**,** 599-620.
GREER, J. B. & WHITCOMB, D. C. 2007. Role of BRCA1 and BRCA2 mutations in pancreatic cancer. *Gut,* 56**,** 601-5.
GROSSMAN, L. & WEI, Q. 1995. DNA repair and epidemiology of basal cell carcinoma. *Clin Chem,* 41**,** 1854-63.
GUPTA, C. & MAZZARA, P. F. 2005. High-grade pancreatic intraepithelial neoplasia in a patient with familial adenomatous polyposis. *Arch Pathol Lab Med,* 129**,** 1398-400.
GUPTA, S., WANG, F., HOLLY, E. A. & BRACCI, P. M. 2010. Risk of pancreatic cancer by alcohol dose, duration, and pattern of consumption, including binge drinking: a population-based study. *Cancer causes & control : CCC,* 21**,** 1047-59.
HABBE, N., LANGER, P., SINA-FREY, M. & BARTSCH, D. K. 2006. Familial pancreatic cancer syndromes. *Endocrinol Metab Clin North Am,* 35**,** 417-30, xi.
HADI, M. Z., COLEMAN, M. A., FIDELIS, K., MOHRENWEISER, H. W. & WILSON, D. M., 3RD 2000. Functional characterization of Ape1 variants identified in the human population. *Nucleic acids research,* 28**,** 3871-9.
HAENO, H., GONEN, M., DAVIS, M. B., HERMAN, J. M., IACOBUZIO-DONAHUE, C. A. & MICHOR, F. 2012. Computational modeling of pancreatic cancer reveals kinetics of metastasis suggesting optimum treatment strategies. *Cell,* 148**,** 362-75.
HAMMOND, E. C. 1964. SMOKING IN RELATION TO MORTALITY AND MORBIDITY. FINDINGS IN FIRST THIRTY-FOUR MONTHS OF FOLLOW-UP IN A PROSPECTIVE STUDY STARTED IN 1959. *J Natl Cancer Inst.,* 32**,** 1161-88.
HANAOKA, T., SUGIMURA, H., NAGURA, K., IHARA, M., LI, X. J., HAMADA, G. S., NISHIMOTO, I., KOWALSKI, L. P., YOKOTA, J. & TSUGANE, S. 2001. hOGG1 exon7 polymorphism and gastric cancer in case-control studies of Japanese Brazilians and non-Japanese Brazilians. *Cancer Lett,* 170**,** 53-61.
HAO, B., WANG, H., ZHOU, K., LI, Y., CHEN, X., ZHOU, G., ZHU, Y., MIAO, X., TAN, W., WEI, Q., LIN, D. & HE, F. 2004. Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. *Cancer Res,* 64**,** 4378-84.
HARNACK, L. J., ANDERSON, K. E., ZHENG, W., FOLSOM, A. R., SELLERS, T. A. & KUSHI, L. H. 1997. Smoking, alcohol, coffee, and tea intake and incidence of cancer of the exocrine pancreas: the Iowa Women's Health Study. *Cancer Epidemiol Biomarkers Prev,* 6**,** 1081-6.

HARRIS, C. C. 1989. Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. *Carcinogenesis,* 10**,** 1563-6.

HASSEN, S., ALI, N. & CHOWDHURY, P. 2012. Molecular signaling mechanisms of apoptosis in hereditary non-polyposis colorectal cancer. *World journal of gastrointestinal pathophysiology,* 3**,** 71-9.

HEARLE, N., SCHUMACHER, V., MENKO, F. H., OLSCHWANG, S., BOARDMAN, L. A., GILLE, J. J., KELLER, J. J., WESTERMAN, A. M., SCOTT, R. J., LIM, W., TRIMBATH, J. D., GIARDIELLO, F. M., GRUBER, S. B., OFFERHAUS, G. J., DE ROOIJ, F. W., WILSON, J. H., HANSMANN, A., MOSLEIN, G., ROYER-POKORA, B., VOGEL, T., PHILLIPS, R. K., SPIGELMAN, A. D. & HOULSTON, R. S. 2006. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clinical cancer research : an official journal of the American Association for Cancer Research,* 12**,** 3209-15.

HECHT, S. S. 1998. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem Res Toxicol,* 11**,** 559-603.

HECHT, S. S. & HOFFMANN, D. 1988. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis,* 9**,** 875-84.

HEINEMANN, V., BOECK, S., HINKE, A., LABIANCA, R. & LOUVET, C. 2008. Meta-analysis of randomized trials: evaluation of benefit from gemcitabine-based combination chemotherapy applied in advanced pancreatic cancer. *BMC Cancer,* 8**,** 82.

HIDALGO, M. 2010. Pancreatic cancer. *N Engl J Med,* 362**,** 1605-17.

HIRAO, T., NELSON, H. H., ASHOK, T. D., WAIN, J. C., MARK, E. J., CHRISTIANI, D. C., WIENCKE, J. K. & KELSEY, K. T. 2001. Tobacco smoke-induced DNA damage and an early age of smoking initiation induce chromosome loss at 3p21 in lung cancer. *Cancer Res,* 61**,** 612-5.

HOFFMANN, D., RIVENSON, A., CHUNG, F. L. & HECHT, S. S. 1991. Nicotine-derived N-nitrosamines (TSNA) and their relevance in tobacco carcinogenesis. *Crit Rev Toxicol,* 21**,** 305-11.

HOULSTON, R. S. & PETO, J. 2004. The search for low-penetrance cancer susceptibility alleles. *Oncogene,* 23**,** 6471-6.

HOWES, N., LERCH, M. M., GREENHALF, W., STOCKEN, D. D., ELLIS, I., SIMON, P., TRUNINGER, K., AMMANN, R., CAVALLINI, G., CHARNLEY, R. M., UOMO, G., DELHAYE, M., SPICAK, J., DRUMM, B., JANSEN, J., MOUNTFORD, R., WHITCOMB, D. C. & NEOPTOLEMOS, J. P. 2004. Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin Gastroenterol Hepatol,* 2**,** 252-61.

HRUBAN, R. H., GOGGINS, M., PARSONS, J. & KERN, S. E. 2000. Progression model for pancreatic cancer. *Clin Cancer Res,* 6**,** 2969-72.

HRUBAN, R. H., WILENTZ, R. E. & MAITRA, A. 2005. Identification and analysis of precursors to invasive pancreatic cancer. *Methods Mol Med,* 103**,** 1-13.

HTTP://WWW.CFF.ORG/ABOUTCFFOUNDATION/NEWSEVENTS/2006NEWSARCHIVE/2711.CFM. 2006. Available: http://www.cff.org/aboutCFFoundation/NewsEvents/2006NewsArchive/2711.cfm.

HU, J. J., SMITH, T. R., MILLER, M. S., MOHRENWEISER, H. W., GOLDEN, A. & CASE, L. D. 2001. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. *Carcinogenesis,* 22**,** 917-22.

HU, Z., MA, H., CHEN, F., WEI, Q. & SHEN, H. 2005. XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. *Cancer Epidemiol Biomarkers Prev,* 14**,** 1810-8.

HUGUET, F., ANDRE, T., HAMMEL, P., ARTRU, P., BALOSSO, J., SELLE, F., DENIAUD-ALEXANDRE, E., RUSZNIEWSKI, P., TOUBOUL, E., LABIANCA, R., DE GRAMONT, A. & LOUVET, C. 2007. Impact of chemoradiotherapy after disease control with chemotherapy in locally advanced pancreatic adenocarcinoma in GERCOR phase II and III studies. *J Clin Oncol,* 25**,** 326-31.

HUNG, R. J., BRENNAN, P., CANZIAN, F., SZESZENIA-DABROWSKA, N., ZARIDZE, D., LISSOWSKA, J., RUDNAI, P., FABIANOVA, E., MATES, D., FORETOVA, L., JANOUT, V., BENCKO, V., CHABRIER, A., BOREL, S., HALL, J. & BOFFETTA, P. 2005a. Large-scale investigation of base excision repair genetic polymorphisms and lung cancer risk in a multicenter study. *J Natl Cancer Inst,* 97**,** 567-76.

HUNG, R. J., HALL, J., BRENNAN, P. & BOFFETTA, P. 2005b. Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *American journal of epidemiology,* 162**,** 925-42.

HUNG, R. J., HALL, J., BRENNAN, P. & BOFFETTA, P. 2005c. Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol,* 162**,** 925-42.

HUSER, N., MICHALSKI, C. W., SCHUSTER, T., FRIESS, H. & KLEEFF, J. 2009. Systematic review and meta-analysis of prophylactic gastroenterostomy for unresectable advanced pancreatic cancer. *Br J Surg,* 96**,** 711-9.

IAKIMOV, A. & ZHELEVA, I. 1991. [Pancreatic cancer. I. The age distribution and location of the tumor process in the gland (a statistical analysis based on autopsy data from the Department of Pathological Anatomy of the Biomedical Research Institute over the 25 years from 1963-1987]. *Khirurgiia (Sofiia),* 44**,** 28-31.

IMYANITOV, E. N., TOGO, A. V. & HANSON, K. P. 2004. Searching for cancer-associated gene polymorphisms: promises and obstacles. *Cancer Lett,* 204**,** 3-14.

IODICE, S., GANDINI, S., MAISONNEUVE, P. & LOWENFELS, A. B. 2008. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. *Langenbecks Arch Surg,* 393**,** 535-45.

IODICE, S., MAISONNEUVE, P., BOTTERI, E., SANDRI, M. T. & LOWENFELS, A. B. 2010. ABO blood group and cancer. *European journal of cancer,* 46**,** 3345-50.

IQBAL, J., RAGONE, A., LUBINSKI, J., LYNCH, H. T., MOLLER, P., GHADIRIAN, P., FOULKES, W. D., ARMEL, S., EISEN, A., NEUHAUSEN, S. L., SENTER, L., SINGER, C. F., AINSWORTH, P., KIM-SING, C., TUNG, N., FRIEDMAN, E., LLACUACHAQUI, M., PING, S. & NAROD, S. A. 2012. The incidence of pancreatic cancer in BRCA1 and BRCA2 mutation carriers. *British journal of cancer,* 107**,** 2005-9.

ITO, H., HAMAJIMA, N., TAKEZAKI, T., MATSUO, K., TAJIMA, K., HATOOKA, S., MITSUDOMI, T., SUYAMA, M., SATO, S. & UEDA, R. 2002. A limited association of OGG1 Ser326Cys polymorphism for adenocarcinoma of the lung. *J Epidemiol,* 12**,** 258-65.

ITO, H., MATSUO, K., HAMAJIMA, N., MITSUDOMI, T., SUGIURA, T., SAITO, T., YASUE, T., LEE, K. M., KANG, D., YOO, K. Y., SATO, S., UEDA, R. & TAJIMA, K. 2004. Gene-environment interactions between the smoking habit and polymorphisms in the DNA repair genes, APE1 Asp148Glu and XRCC1 Arg399Gln, in Japanese lung cancer risk. *Carcinogenesis,* 25**,** 1395-401.

JACKSON, A. D., DALY, L., JACKSON, A. L., KELLEHER, C., MARSHALL, B. C., QUINTON, H. B., FLETCHER, G., HARRINGTON, M., ZHOU, S., MCKONE, E. F., GALLAGHER, C., FOLEY, L. & FITZPATRICK, P. 2011.

Validation and use of a parametric model for projecting cystic fibrosis survivorship beyond observed data: a birth cohort analysis. *Thorax*.

JACOBS, D. R., JR., ADACHI, H., MULDER, I., KROMHOUT, D., MENOTTI, A., NISSINEN, A. & BLACKBURN, H. 1999. Cigarette smoking and mortality risk: twenty-five-year follow-up of the Seven Countries Study. *Arch Intern Med,* 159**,** 733-40.

JACOBS, E. J., CHANOCK, S. J., FUCHS, C. S., LACROIX, A., MCWILLIAMS, R. R., STEPLOWSKI, E., STOLZENBERG-SOLOMON, R. Z., ARSLAN, A. A., BUENO-DE-MESQUITA, H. B., GROSS, M., HELZLSOUER, K., PETERSEN, G., ZHENG, W., AGALLIU, I., ALLEN, N. E., AMUNDADOTTIR, L., BOUTRON-RUAULT, M. C., BURING, J. E., CANZIAN, F., CLIPP, S., DORRONSORO, M., GAZIANO, J. M., GIOVANNUCCI, E. L., HANKINSON, S. E., HARTGE, P., HOOVER, R. N., HUNTER, D. J., JACOBS, K. B., JENAB, M., KRAFT, P., KOOPERBERG, C., LYNCH, S. M., SUND, M., MENDELSOHN, J. B., MOUW, T., NEWTON, C. C., OVERVAD, K., PALLI, D., PEETERS, P. H., RAJKOVIC, A., SHU, X. O., THOMAS, G., TOBIAS, G. S., TRICHOPOULOS, D., VIRTAMO, J., WACTAWSKI-WENDE, J., WOLPIN, B. M., YU, K. & ZELENIUCH-JACQUOTTE, A. Family history of cancer and risk of pancreatic cancer: A pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Int J Cancer*.

JACOBS, E. J., RODRIGUEZ, C., NEWTON, C. C., BAIN, E. B., PATEL, A. V., FEIGELSON, H. S., THUN, M. J. & CALLE, E. E. 2009. Family history of various cancers and pancreatic cancer mortality in a large cohort. *Cancer Causes Control,* 20**,** 1261-9.

JAMES, T. A., SHELDON, D. G., RAJPUT, A., KUVSHINOFF, B. W., JAVLE, M. M., NAVA, H. R., SMITH, J. L. & GIBBS, J. F. 2004. Risk factors associated with earlier age of onset in familial pancreatic carcinoma. *Cancer,* 101**,** 2722-6.

JEURNINK, S. M., VAN EIJCK, C. H., STEYERBERG, E. W., KUIPERS, E. J. & SIERSEMA, P. D. 2007. Stent versus gastrojejunostomy for the palliation of gastric outlet obstruction: a systematic review. *BMC Gastroenterol,* 7**,** 18.

JIAO, L., BONDY, M. L., HASSAN, M. M., WOLFF, R. A., EVANS, D. B., ABBRUZZESE, J. L. & LI, D. 2006a. Selected polymorphisms of DNA repair genes and risk of pancreatic cancer. *Cancer Detect Prev,* 30**,** 284-91.

JIAO, L., BONDY, M. L., HASSAN, M. M., WOLFF, R. A., EVANS, D. B., ABBRUZZESE, J. L. & LI, D. 2006b. Selected polymorphisms of DNA repair genes and risk of pancreatic cancer. *Cancer detection and prevention,* 30**,** 284-91.

JIAO, L., HASSAN, M. M., BONDY, M. L., ABBRUZZESE, J. L., EVANS, D. B. & LI, D. 2007. The XPD Asp312Asn and Lys751Gln polymorphisms, corresponding haplotype, and pancreatic cancer risk. *Cancer Lett,* 245**,** 61-8.

JIAO, L., HASSAN, M. M., BONDY, M. L., WOLFF, R. A., EVANS, D. B., ABBRUZZESE, J. L. & LI, D. 2008a. XRCC2 and XRCC3 gene polymorphism and risk of pancreatic cancer. *The American journal of gastroenterology,* 103**,** 360-7.

JIAO, L., HASSAN, M. M., BONDY, M. L., WOLFF, R. A., EVANS, D. B., ABBRUZZESE, J. L. & LI, D. 2008b. XRCC2 and XRCC3 gene polymorphism and risk of pancreatic cancer. *Am J Gastroenterol,* 103**,** 360-7.

KARMAZANOVSKY, G., FEDOROV, V., KUBYSHKIN, V. & KOTCHATKOV, A. 2005. Pancreatic head cancer: accuracy of CT in determination of resectability. *Abdom Imaging,* 30**,** 488-500.

KATZ, M. H., PISTERS, P. W., EVANS, D. B., SUN, C. C., LEE, J. E., FLEMING, J. B., VAUTHEY, J. N., ABDALLA, E. K., CRANE, C. H., WOLFF, R. A., VARADHACHARY, G. R. & HWANG, R. F. 2008. Borderline resectable pancreatic cancer: the importance of this emerging stage of disease. *J Am Coll Surg,* 206**,** 833-46; discussion 846-8.

KHANNA, K. K. & JACKSON, S. P. 2001. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet,* 27**,** 247-54.

KIMMEY, M. B., BRONNER, M. P., BYRD, D. R. & BRENTNALL, T. A. 2002. Screening and surveillance for hereditary pancreatic cancer. *Gastrointest Endosc,* 56**,** S82-6.

KIMURA, W., NAGAI, H., KURODA, A., MUTO, T. & ESAKI, Y. 1995. Analysis of small cystic lesions of the pancreas. *Int J Pancreatol,* 18**,** 197-206.

KLAPMAN, J. & MALAFA, M. P. 2008. Early detection of pancreatic cancer: why, who, and how to screen. *Cancer Control,* 15**,** 280-7.

KLEIN, A. P., BRUNE, K. A., PETERSEN, G. M., GOGGINS, M., TERSMETTE, A. C., OFFERHAUS, G. J., GRIFFIN, C., CAMERON, J. L., YEO, C. J., KERN, S. & HRUBAN, R. H. 2004. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res,* 64**,** 2634-8.

KLUIJT, I., CATS, A., FOCKENS, P., NIO, Y., GOUMA, D. J. & BRUNO, M. J. 2009. Atypical familial presentation of FAMMM syndrome with a high incidence of pancreatic cancer: case finding of asymptomatic individuals by EUS surveillance. *J Clin Gastroenterol,* 43**,** 853-7.

KNEUERTZ, P. J., PATEL, S. H., CHU, C. K., MAITHEL, S. K., SARMIENTO, J. M., DELMAN, K. A., STALEY, C. A., 3RD & KOOBY, D. A. 2011. Effects of perioperative red blood cell transfusion on disease recurrence and survival after pancreaticoduodenectomy for ductal adenocarcinoma. *Annals of surgical oncology,* 18**,** 1327-34.

KOBAYASHI, N., SUGIMORI, K., SHIMAMURA, T., HOSONO, K., WATANABE, S., KATO, S., UEDA, M., ENDO, I., INAYAMA, Y., MAEDA, S., NAKAJIMA, A. & KUBOTA, K. 2012. Endoscopic ultrasonographic findings predict the risk of carcinoma in branch duct intraductal papillary mucinous neoplasms of the pancreas. *Pancreatology : official journal of the International Association of Pancreatology,* 12**,** 141-5.

KOHNO, T., SHINMURA, K., TOSAKA, M., TANI, M., KIM, S. R., SUGIMURA, H., NOHMI, T., KASAI, H. & YOKOTA, J. 1998. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene,* 16**,** 3219-25.

KOLODNER, R. D. & MARSISCHKY, G. T. 1999. Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev,* 9**,** 89-96.

KRAEMER, K. H., LEVY, D. D., PARRIS, C. N., GOZUKARA, E. M., MORIWAKI, S., ADELBERG, S. & SEIDMAN, M. M. 1994. Xeroderma pigmentosum and related disorders: examining the linkage between defective DNA repair and cancer. *The Journal of investigative dermatology,* 103**,** 96S-101S.

KRUK, P. A., RAMPINO, N. J. & BOHR, V. A. 1995. DNA damage and repair in telomeres: relation to aging. *Proc Natl Acad Sci U S A,* 92**,** 258-62.

LAI, C. & SHIELDS, P. G. 1999. The role of interindividual variation in human carcinogenesis. *J Nutr,* 129**,** 552S-555S.

LAI, E. C., MOK, F. P., FAN, S. T., LO, C. M., CHU, K. M., LIU, C. L. & WONG, J. 1994. Preoperative endoscopic drainage for malignant obstructive jaundice. *The British journal of surgery,* 81**,** 1195-8.

LATCHFORD, A., GREENHALF, W., VITONE, L. J., NEOPTOLEMOS, J. P., LANCASTER, G. A. & PHILLIPS, R. K. 2006. Peutz-Jeghers syndrome and screening for pancreatic cancer. *Br J Surg,* 93**,** 1446-55.

LE MARCHAND, L., DONLON, T., LUM-JONES, A., SEIFRIED, A. & WILKENS, L. R. 2002. Association of the hOGG1 Ser326Cys polymorphism with lung cancer risk. *Cancer Epidemiol Biomarkers Prev,* 11**,** 409-12.

LEFROU, L., GODART, B., DE MURET, A., SCOTTO, B. & DORVAL, E. 2006. [Germline tp53 neomutation in a patient with li-fraumeni syndrome and pancreatic adenocarcinoma]. *Gastroenterol Clin Biol,* 30**,** 484-6.

LEWIS, R., DREBIN, J. A., CALLERY, M. P., FRAKER, D., KENT, T. S., GATES, J. & VOLLMER, C. M., JR. 2013. A contemporary analysis of survival for resected pancreatic ductal adenocarcinoma. *HPB : the official journal of the International Hepato Pancreato Biliary Association,* 15**,** 49-60.

LI, D. 2002. Molecular Epidemiology. *In:* EVANS, D. B., PISTERS, P. W. T. & ABBRUZZESE, J. L. (eds.) *Pancreatic Cancer.* New York: Springer-Verlag.

LI, D., FRAZIER, M., EVANS, D. B., HESS, K. R., CRANE, C. H., JIAO, L. & ABBRUZZESE, J. L. 2006a. Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J Clin Oncol,* 24**,** 1720-8.

LI, D., JIAO, L., LI, Y., DOLL, M. A., HEIN, D. W., BONDY, M. L., EVANS, D. B., WOLFF, R. A., LENZI, R., PISTERS, P. W., ABBRUZZESE, J. L. & HASSAN, M. M. 2006b. Polymorphisms of cytochrome P4501A2 and N-acetyltransferase genes, smoking, and risk of pancreatic cancer. *Carcinogenesis,* 27**,** 103-11.

LI, D., LI, Y., JIAO, L., CHANG, D. Z., BEINART, G., WOLFF, R. A., EVANS, D. B., HASSAN, M. M. & ABBRUZZESE, J. L. 2007a. Effects of base excision repair gene polymorphisms on pancreatic cancer survival. *Int J Cancer,* 120**,** 1748-54.

LI, D., LI, Y., JIAO, L., CHANG, D. Z., BEINART, G., WOLFF, R. A., EVANS, D. B., HASSAN, M. M. & ABBRUZZESE, J. L. 2007b. Effects of base excision repair gene polymorphisms on pancreatic cancer survival. *International journal of cancer. Journal international du cancer,* 120**,** 1748-54.

LI, D., LIU, H., JIAO, L., CHANG, D. Z., BEINART, G., WOLFF, R. A., EVANS, D. B., HASSAN, M. M. & ABBRUZZESE, J. L. 2006c. Significant effect of homologous recombination DNA repair gene polymorphisms on pancreatic cancer survival. *Cancer research,* 66**,** 3323-30.

LI, D., MORRIS, J. S., LIU, J., HASSAN, M. M., DAY, R. S., BONDY, M. L. & ABBRUZZESE, J. L. 2009. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. *Jama,* 301**,** 2553-62.

LI, D., XIE, K., WOLFF, R. & ABBRUZZESE, J. L. 2004. Pancreatic cancer. *Lancet,* 363**,** 1049-57.

LI, F. P. 1990. Familial cancer syndromes and clusters. *Curr Probl Cancer,* 14**,** 73-114.

LILLEMOE, K. D. 1995. Current management of pancreatic carcinoma. *Annals of surgery,* 221**,** 133-48.

LIM, J. E., CHIEN, M. W. & EARLE, C. C. 2003. Prognostic factors following curative resection for pancreatic adenocarcinoma: a population-based, linked database analysis of 396 patients. *Annals of surgery,* 237**,** 74-85.

LINDAHL, T. & WOOD, R. D. 1999. Quality control by DNA repair. *Science,* 286**,** 1897-905.

LOCHAN, R., DALY, A. K., REEVES, H. L. & CHARNLEY, R. M. 2008. Genetic susceptibility in pancreatic ductal adenocarcinoma. *Br J Surg,* 95**,** 22-32.

LOUVET, C., LABIANCA, R., HAMMEL, P., LLEDO, G., ZAMPINO, M. G., ANDRE, T., ZANIBONI, A., DUCREUX, M., AITINI, E., TAIEB, J., FAROUX, R., LEPERE, C. & DE GRAMONT, A. 2005. Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. *J Clin Oncol,* 23**,** 3509-16.

LOW, S. K., KUCHIBA, A., ZEMBUTSU, H., SAITO, A., TAKAHASHI, A., KUBO, M., DAIGO, Y., KAMATANI, N., CHIKU, S., TOTSUKA, H., OHNAMI, S., HIROSE, H., SHIMADA, K., OKUSAKA, T., YOSHIDA, T., NAKAMURA, Y. & SAKAMOTO, H. 2010. Genome-wide association study of pancreatic cancer in Japanese population. *PloS one,* 5**,** e11824.

LOWENFELS, A. B., MAISONNEUVE, P., DIMAGNO, E. P., ELITSUR, Y., GATES, L. K., JR., PERRAULT, J. & WHITCOMB, D. C. 1997. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst,* 89**,** 442-6.

LOWENFELS, A. B., MAISONNEUVE, P., WHITCOMB, D. C., LERCH, M. M. & DIMAGNO, E. P. 2001. Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. *Jama,* 286**,** 169-70.

LU, A. L., LI, X., GU, Y., WRIGHT, P. M. & CHANG, D. Y. 2001. Repair of oxidative DNA damage: mechanisms and functions. *Cell Biochem Biophys,* 35**,** 141-70.

LUBINSKI, J., PHELAN, C. M., GHADIRIAN, P., LYNCH, H. T., GARBER, J., WEBER, B., TUNG, N., HORSMAN, D., ISAACS, C., MONTEIRO, A. N., SUN, P. & NAROD, S. A. 2004. Cancer variation associated with the position of the mutation in the BRCA2 gene. *Fam Cancer,* 3**,** 1-10.

LUCENTEFORTE, E., LA VECCHIA, C., SILVERMAN, D., PETERSEN, G. M., BRACCI, P. M., JI, B. T., BOSETTI, C., LI, D., GALLINGER, S., MILLER, A. B., BUENO-DE-MESQUITA, H. B., TALAMINI, R., POLESEL, J., GHADIRIAN, P., BAGHURST, P. A., ZATONSKI, W., FONTHAM, E., BAMLET, W. R., HOLLY, E. A., GAO, Y. T., NEGRI, E., HASSAN, M., COTTERCHIO, M., SU, J., MAISONNEUVE, P., BOFFETTA, P. & DUELL, E. J. 2012. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO,* 23**,** 374-82.

LUNN, R. M., LANGLOIS, R. G., HSIEH, L. L., THOMPSON, C. L. & BELL, D. A. 1999. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. *Cancer Res,* 59**,** 2557-61.

LYNCH, H. T., BRAND, R. E., HOGG, D., DETERS, C. A., FUSARO, R. M., LYNCH, J. F., LIU, L., KNEZETIC, J., LASSAM, N. J., GOGGINS, M. & KERN, S. 2002. Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families: the familial atypical mole melanoma-pancreatic carcinoma syndrome. *Cancer,* 94**,** 84-96.

LYNCH, H. T. & FUSARO, R. M. 1991. Pancreatic cancer and the familial atypical multiple mole melanoma (FAMMM) syndrome. *Pancreas,* 6**,** 127-31.

MAISONNEUVE, P., FITZSIMMONS, S. C., NEGLIA, J. P., CAMPBELL, P. W., 3RD & LOWENFELS, A. B. 2003. Cancer risk in nontransplanted and transplanted cystic fibrosis patients: a 10-year study. *J Natl Cancer Inst,* 95**,** 381-7.

MAISONNEUVE, P., MARSHALL, B. C. & LOWENFELS, A. B. 2007. Risk of pancreatic cancer in patients with cystic fibrosis. *Gut,* 56**,** 1327-8.

MANSFIELD, S. D., SCOTT, J., OPPONG, K., RICHARDSON, D. L., SEN, G., JAQUES, B. C., MANAS, D. M. & CHARNLEY, R. M. 2008. Comparison of multislice computed tomography and endoscopic ultrasonography with operative and histological findings in suspected pancreatic and periampullary malignancy. *Br J Surg,* 95**,** 1512-20.

MANSFIELD, S. D., SEN, G., OPPONG, K., JACQUES, B. C., O'SUILLEABHAIN, C. B., MANAS, D. M. & CHARNLEY, R. M. 2006. Increase in serum bilirubin levels in obstructive jaundice secondary to pancreatic and periampullary malignancy--implications for timing of resectional surgery and use of biliary drainage. *HPB (Oxford),* 8**,** 442-5.

MARTIN-LOPEZ, J. V. & FISHEL, R. 2013. The mechanism of mismatch repair and the functional analysis of mismatch repair defects in Lynch syndrome. *Familial cancer*.

MATULLO, G., GUARRERA, S., CARTURAN, S., PELUSO, M., MALAVEILLE, C., DAVICO, L., PIAZZA, A. & VINEIS, P. 2001a. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. *Int J Cancer,* 92**,** 562-7.

MATULLO, G., PALLI, D., PELUSO, M., GUARRERA, S., CARTURAN, S., CELENTANO, E., KROGH, V., MUNNIA, A., TUMINO, R., POLIDORO, S., PIAZZA, A. & VINEIS, P. 2001b. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis,* 22**,** 1437-45.

MCCARTY, M. F. 2001. Mortality from Western cancers rose dramatically among African-Americans during the 20th century: are dietary animal products to blame? *Med Hypotheses,* 57**,** 169-74.

MCCREDIE, M., COX, B. & STEWART, J. H. 2000. Smoking-related Cancers in Maori and non-Maori in New Zealand, 1974-1993: Fewer Bladder Cancers among Maori. *Asian Pac J Cancer Prev,* 1**,** 221-225.

MCFAUL, C. D., GREENHALF, W., EARL, J., HOWES, N., NEOPTOLEMOS, J. P., KRESS, R., SINA-FREY, M., RIEDER, H., HAHN, S. & BARTSCH, D. K. 2006. Anticipation in familial pancreatic cancer. *Gut,* 55**,** 252-8.

MCNULTY, N. J., FRANCIS, I. R., PLATT, J. F., COHAN, R. H., KOROBKIN, M. & GEBREMARIAM, A. 2001. Multi--detector row helical CT of the pancreas: effect of contrast-enhanced multiphasic imaging on enhancement of the pancreas, peripancreatic vasculature, and pancreatic adenocarcinoma. *Radiology,* 220**,** 97-102.

MCWILLIAMS, R., HIGHSMITH, W. E., RABE, K. G., DE ANDRADE, M., TORDSEN, L. A., HOLTEGAARD, L. M. & PETERSEN, G. M. 2005a. Cystic fibrosis transmembrane regulator gene carrier status is a risk factor for young onset pancreatic adenocarcinoma. *Gut,* 54**,** 1661-2.

MCWILLIAMS, R. R., PETERSEN, G. M., RABE, K. G., HOLTEGAARD, L. M., LYNCH, P. J., BISHOP, M. D. & HIGHSMITH, W. E., JR. 2010. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and risk for pancreatic adenocarcinoma. *Cancer,* 116**,** 203-9.

MCWILLIAMS, R. R., RABE, K. G., OLSWOLD, C., DE ANDRADE, M. & PETERSEN, G. M. 2005b. Risk of malignancy in first-degree relatives of patients with pancreatic carcinoma. *Cancer,* 104**,** 388-94.

MEGER, M., RICHTER, E., ZWICKENPFLUG, W., OEHLMANN, C., HARGADEN, M. B., YI, A. R. & VESELL, E. S. 1999. Metabolism and disposition of 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone (NNK) in rhesus monkeys. *Drug metabolism and disposition: the biological fate of chemicals,* 27**,** 471-8.

MICHAELS, P. J., BRACHTEL, E. F., BOUNDS, B. C., BRUGGE, W. R. & PITMAN, M. B. 2006. Intraductal papillary mucinous neoplasm of the pancreas: cytologic features predict histologic grade. *Cancer,* 108**,** 163-73.

MICHALSKI, C. W., KLEEFF, J., WENTE, M. N., DIENER, M. K., BUCHLER, M. W. & FRIESS, H. 2007. Systematic review and meta-analysis of standard and extended lymphadenectomy in pancreaticoduodenectomy for pancreatic cancer. *The British journal of surgery,* 94**,** 265-73.

MICHAUD, D. S., VRIELING, A., JIAO, L., MENDELSOHN, J. B., STEPLOWSKI, E., LYNCH, S. M., WACTAWSKI-WENDE, J., ARSLAN, A. A., BAS BUENO-DE-MESQUITA, H., FUCHS, C. S., GROSS, M., HELZLSOUER, K., JACOBS, E. J., LACROIX, A., PETERSEN, G., ZHENG, W., ALLEN, N., AMMUNDADOTTIR, L., BERGMANN, M. M., BOFFETTA, P., BURING, J. E., CANZIAN, F., CHANOCK, S. J., CLAVEL-CHAPELON, F., CLIPP, S., FREIBERG, M. S., MICHAEL GAZIANO, J., GIOVANNUCCI, E. L., HANKINSON, S., HARTGE, P., HOOVER, R. N., ALLAN HUBBELL, F., HUNTER, D. J., HUTCHINSON, A., JACOBS, K., KOOPERBERG, C., KRAFT, P., MANJER, J., NAVARRO, C., PEETERS, P. H., SHU, X. O., STEVENS, V., THOMAS, G., TJONNELAND, A., TOBIAS, G. S., TRICHOPOULOS, D., TUMINO, R., VINEIS, P., VIRTAMO, J., WALLACE, R., WOLPIN, B. M., YU, K., ZELENIUCH-JACQUOTTE, A. & STOLZENBERG-SOLOMON, R. Z. 2010. Alcohol intake and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium (PanScan). *Cancer causes & control : CCC,* 21**,** 1213-25.

MISRA, R. R., RATNASINGHE, D., TANGREA, J. A., VIRTAMO, J., ANDERSEN, M. R., BARRETT, M., TAYLOR, P. R. & ALBANES, D. 2003. Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer among male smokers in Finland. *Cancer Lett,* 191**,** 171-8.

MITCHELL, R. J., BREWSTER, D., CAMPBELL, H., PORTEOUS, M. E., WYLLIE, A. H., BIRD, C. C. & DUNLOP, M. G. 2004. Accuracy of reporting of family history of colorectal cancer. *Gut,* 53**,** 291-5.

MOORE, M. J., GOLDSTEIN, D., HAMM, J., FIGER, A., HECHT, J. R., GALLINGER, S., AU, H. J., MURAWA, P., WALDE, D., WOLFF, R. A., CAMPOS, D., LIM, R., DING, K., CLARK, G., VOSKOGLOU-NOMIKOS, T., PTASYNSKI, M. & PARULEKAR, W. 2007. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol,* 25**,** 1960-6.

MORGAN, R. G. & WORMSLEY, K. G. 1977. Progress report. Cancer of the pancreas. *Gut,* 18**,** 580-92.

MOSKALUK, C. A., HRUBAN, R. H. & KERN, S. E. 1997. p16 and K-ras gene mutations in the intraductal precursors of human pancreatic adenocarcinoma. *Cancer Res,* 57**,** 2140-3.

MOSS, A. C., MORRIS, E., LEYDEN, J. & MACMATHUNA, P. 2007a. Do the benefits of metal stents justify the costs? A systematic review and meta-analysis of trials comparing endoscopic stents for malignant biliary obstruction. *European journal of gastroenterology & hepatology,* 19**,** 1119-24.

MOSS, A. C., MORRIS, E., LEYDEN, J. & MACMATHUNA, P. 2007b. Malignant distal biliary obstruction: a systematic review and meta-analysis of endoscopic and surgical bypass results. *Cancer treatment reviews,* 33**,** 213-21.

MOSSNER, J. 2010. What's New in Therapy of Pancreatic Cancer? *Dig Dis,* 28**,** 679-83.

MURFF, H. J., SPIGEL, D. R. & SYNGAL, S. 2004. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. *Jama,* 292**,** 1480-9.

MURPHY, K. M., BRUNE, K. A., GRIFFIN, C., SOLLENBERGER, J. E., PETERSEN, G. M., BANSAL, R., HRUBAN, R. H. & KERN, S. E. 2002. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. *Cancer Res,* 62**,** 3789-93.

NAKAKURA, N. K. & YEO, C. J. 2006. Periampullary and pancreatic cancer. *In:* BLUMGART, L. H. (ed.) *Surgery of the Liver, Biliary Tract and Pancreas.* Philadelphia: Saunders Elsevier.

NEGLIA, J. P., FITZSIMMONS, S. C., MAISONNEUVE, P., SCHONI, M. H., SCHONI-AFFOLTER, F., COREY, M. & LOWENFELS, A. B. 1995. The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. *N Engl J Med,* 332**,** 494-9.

NELSON, H. H., KELSEY, K. T., MOTT, L. A. & KARAGAS, M. R. 2002. The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. *Cancer Res,* 62**,** 152-5.

NEOPTOLEMOS, J. P., CUNNINGHAM, D., FRIESS, H., BASSI, C., STOCKEN, D. D., TAIT, D. M., DUNN, J. A., DERVENIS, C., LACAINE, F., HICKEY, H., RARATY, M. G., GHANEH, P. & BUCHLER, M. W. 2003a. Adjuvant therapy in pancreatic cancer: historical and current perspectives. *Ann Oncol,* 14**,** 675-92.

NEOPTOLEMOS, J. P., RARATY, M. G., GHANEH, P., HICKEY, H., STOCKEN, D. D., DUNN, J. A., FRIESS, H. & BUCHLER, M. W. 2003b. [Adjuvant and additive therapy for cancer of the pancreas]. *Chirurg,* 74**,** 191-201.

NIEDERGETHMANN, M., REXIN, M., HILDENBRAND, R., KNOB, S., STURM, J. W., RICHTER, A. & POST, S. 2002. Prognostic implications of routine, immunohistochemical, and molecular staging in resectable pancreatic adenocarcinoma. *Am J Surg Pathol,* 26**,** 1578-87.

NILSEN, T. I. & VATTEN, L. J. 2000. A prospective study of lifestyle factors and the risk of pancreatic cancer in Nord-Trondelag, Norway. *Cancer Causes Control,* 11**,** 645-52.

NIO, Y., DONG, M., IGUCHI, C., YAMASAWA, K., TOGA, T., ITAKURA, M. & TAMURA, K. 2001. Expression of Bcl-2 and p53 protein in resectable invasive ductal carcinoma of the pancreas: effects on clinical outcome and efficacy of adjuvant chemotherapy. *J Surg Oncol,* 76**,** 188-96.

NIO, Y., DONG, M., UEGAKI, K., HIRAHARA, N., MINARI, Y., SASAKI, S., TAKAMURA, M., IGUCHI, C. & TAMURA, K. 1998. p53 expression affects the efficacy of adjuvant chemotherapy after resection of invasive ductal carcinoma of the pancreas. *Anticancer Res,* 18**,** 3773-9.

NYAGA, S. G., LOHANI, A. & EVANS, M. K. 2008. Deficient repair of 8-hydroxyguanine in the BxPC-3 pancreatic cancer cell line. *Biochemical and biophysical research communications,* 376**,** 336-40.

OCKENGA, J., VOGEL, A., TEICH, N., KEIM, V., MANNS, M. P. & STRASSBURG, C. P. 2003. UDP glucuronosyltransferase (UGT1A7) gene polymorphisms increase the risk of chronic pancreatitis and pancreatic cancer. *Gastroenterology,* 124**,** 1802-8.

OETTLE, H. 2003. [Adjuvant treatment of pancreatic cancer]. *Zentralbl Chir,* 128**,** 411-8.

OGAWA, H., ITOH, S., IKEDA, M., SUZUKI, K. & NAGANAWA, S. 2008. Intraductal papillary mucinous neoplasm of the pancreas: assessment of the likelihood of invasiveness with multisection CT. *Radiology,* 248**,** 876-86.

OHNO, E., ITOH, A., KAWASHIMA, H., ISHIKAWA, T., MATSUBARA, H., ITOH, Y., NAKAMURA, Y., HIRAMATSU, T., NAKAMURA, M., MIYAHARA, R., OHMIYA, N., ISHIGAMI, M., KATANO, Y., GOTO, H. & HIROOKA, Y. 2012. Malignant transformation of branch duct-type intraductal papillary mucinous neoplasms of the pancreas based on contrast-enhanced endoscopic ultrasonography morphological changes: focus on malignant transformation of intraductal papillary mucinous neoplasm itself. *Pancreas,* 41**,** 855-62.

OKAZAKI, T., JIAO, L., CHANG, P., EVANS, D. B., ABBRUZZESE, J. L. & LI, D. 2008. Single-nucleotide polymorphisms of DNA damage response genes are associated with overall survival in patients with pancreatic cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* 14**,** 2042-8.

OLGYAI, G. & OLAH, A. 2007. [Endoscopic stent or surgical bypass? A review and evidence-based comparison of palliative procedures in inoperable pancreas tumours]. *Magy Seb,* 60**,** 239-42.

ORR, R. K. 2010. Outcomes in pancreatic cancer surgery. *The Surgical clinics of North America,* 90**,** 219-34.

OSVALDT, A. B., WENDT, L. R., BERSCH, V. P., BACKES, A. N., DE CASSIA, A. S. R., EDELWEISS, M. I. & ROHDE, L. 2006. Pancreatic intraepithelial neoplasia and ductal adenocarcinoma induced by DMBA in mice. *Surgery,* 140**,** 803-9.

PARENT, M. E., GHADIRIAN, P., LACROIX, A. & PERRET, C. 1997. The reliability of recollections of family history: implications for the medical provider. *J Cancer Educ,* 12**,** 114-20.

PARK, J., CHEN, L., TOCKMAN, M. S., ELAHI, A. & LAZARUS, P. 2004. The human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) DNA repair enzyme and its association with lung cancer risk. *Pharmacogenetics,* 14**,** 103-9.

PARKIN, D. M., BRAY, F., FERLAY, J. & PISANI, P. 2005. Global cancer statistics, 2002. *CA Cancer J Clin,* 55**,** 74-108.

PARSONS, R., MYEROFF, L. L., LIU, B., WILLSON, J. K., MARKOWITZ, S. D., KINZLER, K. W. & VOGELSTEIN, B. 1995. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res,* 55**,** 5548-50.

PAWLIK, T. M., LAHERU, D., HRUBAN, R. H., COLEMAN, J., WOLFGANG, C. L., CAMPBELL, K., ALI, S., FISHMAN, E. K., SCHULICK, R. D. & HERMAN, J. M. 2008. Evaluating the impact of a single-day multidisciplinary clinic on the management of pancreatic cancer. *Ann Surg Oncol,* 15**,** 2081-8.

PEDDI, P. F., LUBNER, S., MCWILLIAMS, R., TAN, B. R., PICUS, J., SORSCHER, S. M., SURESH, R., LOCKHART, A. C., WANG, J., MENIAS, C., GAO, F., LINEHAN, D. & WANG-GILLAM, A. 2012. Multi-institutional experience with FOLFIRINOX in pancreatic adenocarcinoma. *JOP : Journal of the pancreas,* 13**,** 497-501.

PERMUTH-WEY, J. & EGAN, K. M. 2008. Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. *Fam Cancer*.

PETERSEN, G. M., AMUNDADOTTIR, L., FUCHS, C. S., KRAFT, P., STOLZENBERG-SOLOMON, R. Z., JACOBS, K. B., ARSLAN, A. A., BUENO-DE-MESQUITA, H. B., GALLINGER, S., GROSS, M., HELZLSOUER, K., HOLLY, E. A., JACOBS, E. J., KLEIN, A. P., LACROIX, A., LI, D., MANDELSON, M. T., OLSON, S. H., RISCH, H. A., ZHENG, W., ALBANES, D., BAMLET, W. R., BERG, C. D., BOUTRON-RUAULT, M. C., BURING, J. E., BRACCI, P. M., CANZIAN, F., CLIPP, S., COTTERCHIO,

M., DE ANDRADE, M., DUELL, E. J., GAZIANO, J. M., GIOVANNUCCI, E. L., GOGGINS, M., HALLMANS, G., HANKINSON, S. E., HASSAN, M., HOWARD, B., HUNTER, D. J., HUTCHINSON, A., JENAB, M., KAAKS, R., KOOPERBERG, C., KROGH, V., KURTZ, R. C., LYNCH, S. M., MCWILLIAMS, R. R., MENDELSOHN, J. B., MICHAUD, D. S., PARIKH, H., PATEL, A. V., PEETERS, P. H., RAJKOVIC, A., RIBOLI, E., RODRIGUEZ, L., SEMINARA, D., SHU, X. O., THOMAS, G., TJONNELAND, A., TOBIAS, G. S., TRICHOPOULOS, D., VAN DEN EEDEN, S. K., VIRTAMO, J., WACTAWSKI-WENDE, J., WANG, Z., WOLPIN, B. M., YU, H., YU, K., ZELENIUCH-JACQUOTTE, A., FRAUMENI, J. F., JR., HOOVER, R. N., HARTGE, P. & CHANOCK, S. J. 2010. A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. *Nature genetics,* 42**,** 224-8.

PETERSEN, G. M., DE ANDRADE, M., GOGGINS, M., HRUBAN, R. H., BONDY, M., KORCZAK, J. F., GALLINGER, S., LYNCH, H. T., SYNGAL, S., RABE, K. G., SEMINARA, D. & KLEIN, A. P. 2006. Pancreatic cancer genetic epidemiology consortium. *Cancer Epidemiol Biomarkers Prev,* 15**,** 704-10.

PETERSEN, G. M. & HRUBAN, R. H. 2003. Familial Pancreatic Cancer: Where Are We in 2003? *Journal of the National Cancer Institute,* 95**,** 180-181.

PHILLIPS, A. R., LAWES, C. M., COOPER, G. J. & WINDSOR, J. A. 2002. Ethnic disparity of pancreatic cancer in New Zealand. *Int J Gastrointest Cancer,* 31**,** 137-45.

POGUE-GEILE, K. L., CHEN, R., BRONNER, M. P., CRNOGORAC-JURCEVIC, T., MOYES, K. W., DOWEN, S., OTEY, C. A., CRISPIN, D. A., GEORGE, R. D., WHITCOMB, D. C. & BRENTNALL, T. A. 2006. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. *PLoS Med,* 3**,** e516.

POPANDA, O., SCHATTENBERG, T., PHONG, C. T., BUTKIEWICZ, D., RISCH, A., EDLER, L., KAYSER, K., DIENEMANN, H., SCHULZ, V., DRINGS, P., BARTSCH, H. & SCHMEZER, P. 2004. Specific combinations of DNA repair gene variants and increased risk for non-small cell lung cancer. *Carcinogenesis,* 25**,** 2433-41.

POUR, P. M. & RIVENSON, A. 1989. Induction of a mixed ductal-squamous-islet cell carcinoma in a rat treated with a tobacco-specific carcinogen. *Am J Pathol,* 134**,** 627-31.

PROKOPCZYK, B., HOFFMANN, D., BOLOGNA, M., CUNNINGHAM, A. J., TRUSHIN, N., AKERKAR, S., BOYIRI, T., AMIN, S., DESAI, D., COLOSIMO, S., PITTMAN, B., LEDER, G., RAMADANI, M., HENNE-BRUNS, D., BEGER, H. G. & EL-BAYOUMY, K. 2002. Identification of tobacco-derived compounds in human pancreatic juice. *Chem Res Toxicol,* 15**,** 677-85.

QUINN MJ, BABB PJ, KIRBY L & J., J. 2000. Registrations of cancer diagnosed in 1994-97, England and Wales. *Health Statistics Quarterly,* 7**,** 71-82.

RAFIQUE, A., FREEMAN, S. & CARROLL, N. 2007. A clinical algorithm for the assessment of pancreatic lesions: utilization of 16- and 64-section multidetector CT and endoscopic ultrasound. *Clinical radiology,* 62**,** 1142-53.

RAIMONDI, S., MAISONNEUVE, P., LOHR, J. M. & LOWENFELS, A. B. 2007. Early onset pancreatic cancer: evidence of a major role for smoking and genetic factors. *Cancer Epidemiol Biomarkers Prev,* 16**,** 1894-7.

RANDERATH, E., MITTAL, D. & RANDERATH, K. 1988. Tissue distribution of covalent DNA damage in mice treated dermally with cigarette 'tar': preference for lung and heart DNA. *Carcinogenesis,* 9**,** 75-80.
RANDERATH, E. & RANDERATH, K. 1993. Monitoring tobacco smoke-induced DNA damage by 32P-postlabelling. *IARC Sci Publ***,** 305-14.
RATNASINGHE, D. L., YAO, S. X., FORMAN, M., QIAO, Y. L., ANDERSEN, M. R., GIFFEN, C. A., EROZAN, Y., TOCKMAN, M. S. & TAYLOR, P. R. 2003. Gene-environment interactions between the codon 194 polymorphism of XRCC1 and antioxidants influence lung cancer risk. *Anticancer Res,* 23**,** 627-32.
REDDY, R. P., SMYRK, T. C., ZAPIACH, M., LEVY, M. J., PEARSON, R. K., CLAIN, J. E., FARNELL, M. B., SARR, M. G. & CHARI, S. T. 2004. Pancreatic mucinous cystic neoplasm defined by ovarian stroma: demographics, clinical features, and prevalence of cancer. *Clin Gastroenterol Hepatol,* 2**,** 1026-31.
RIALL, T. S. 2009. What is the effect of age on pancreatic resection? *Advances in surgery,* 43**,** 233-49.
RICHIE, J. P., JR., CARMELLA, S. G., MUSCAT, J. E., SCOTT, D. G., AKERKAR, S. A. & HECHT, S. S. 1997. Differences in the urinary metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in black and white smokers. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology,* 6**,** 783-90.
RISCH, H. A., MCLAUGHLIN, J. R., COLE, D. E., ROSEN, B., BRADLEY, L., FAN, I., TANG, J., LI, S., ZHANG, S., SHAW, P. A. & NAROD, S. A. 2006. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst,* 98**,** 1694-706.
RISINGER, J. I., BARRETT, J. C., WATSON, P., LYNCH, H. T. & BOYD, J. 1996. Molecular genetic evidence of the occurrence of breast cancer as an integral tumor in patients with the hereditary nonpolyposis colorectal carcinoma syndrome. *Cancer,* 77**,** 1836-43.
RIVENSON, A., HOFFMANN, D., PROKOPCZYK, B., AMIN, S. & HECHT, S. S. 1988. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived N-nitrosamines. *Cancer Res,* 48**,** 6912-7.
RODRIGUEZ, S., GAUNT, T. R. & DAY, I. N. 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol,* 169**,** 505-14.
ROSEWICZ, S. & WIEDENMANN, B. 1997. Pancreatic carcinoma. *Lancet,* 349**,** 485-9.
RUBENSTEIN, J. H., SCHEIMAN, J. M. & ANDERSON, M. A. 2007. A clinical and economic evaluation of endoscopic ultrasound for patients at risk for familial pancreatic adenocarcinoma. *Pancreatology,* 7**,** 514-25.
RULYAK, S. J., BRENTNALL, T. A., LYNCH, H. T. & AUSTIN, M. A. 2003a. Characterization of the neoplastic phenotype in the familial atypical multiple-mole melanoma-pancreatic carcinoma syndrome. *Cancer,* 98**,** 798-804.
RULYAK, S. J., KIMMEY, M. B., VEENSTRA, D. L. & BRENTNALL, T. A. 2003b. Cost-effectiveness of pancreatic cancer screening in familial pancreatic cancer kindreds. *Gastrointest Endosc,* 57**,** 23-9.
RULYAK, S. J., LOWENFELS, A. B., MAISONNEUVE, P. & BRENTNALL, T. A. 2003c. Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. *Gastroenterology,* 124**,** 1292-9.
RUSSO, M. T., DE LUCA, G., DEGAN, P., PARLANTI, E., DOGLIOTTI, E., BARNES, D. E., LINDAHL, T., YANG, H., MILLER, J. H. & BIGNAMI, M.

2004. Accumulation of the oxidative base lesion 8-hydroxyguanine in DNA of tumor-prone mice defective in both the Myh and Ogg1 DNA glycosylases. *Cancer Res,* 64**,** 4411-4.

SALVIA, R., FERNANDEZ-DEL CASTILLO, C., BASSI, C., THAYER, S. P., FALCONI, M., MANTOVANI, W., PEDERZOLI, P. & WARSHAW, A. L. 2004. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. *Ann Surg,* 239**,** 678-85; discussion 685-7.

SCHMIDT, C. M., TURRINI, O., PARIKH, P., HOUSE, M. G., ZYROMSKI, N. J., NAKEEB, A., HOWARD, T. J., PITT, H. A. & LILLEMOE, K. D. 2010. Effect of hospital volume, surgeon experience, and surgeon volume on patient outcomes after pancreaticoduodenectomy: a single-institution experience. *Archives of surgery,* 145**,** 634-40.

SCHNEIDER, J., CLASSEN, V., BERNGES, U. & PHILIPP, M. 2005. XRCC1 polymorphism and lung cancer risk in relation to tobacco smoking. *International journal of molecular medicine,* 16**,** 709-16.

SCHNEIDER, J., CLASSEN, V. & HELMIG, S. 2008. XRCC1 polymorphism and lung cancer risk. *Expert Rev Mol Diagn,* 8**,** 761-80.

SCHNELLDORFER, T., SARR, M. G., NAGORNEY, D. M., ZHANG, L., SMYRK, T. C., QIN, R., CHARI, S. T. & FARNELL, M. B. 2008. Experience with 208 resections for intraductal papillary mucinous neoplasm of the pancreas. *Arch Surg,* 143**,** 639-46; discussion 646.

SCHULZE, J., RICHTER, E., BINDER, U. & ZWICKENPFLUG, W. 1992. Biliary excretion of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the rat. *Carcinogenesis,* 13**,** 1961-5.

SHAH, K. R., BOLAND, C. R., PATEL, M., THRASH, B. & MENTER, A. 2013. Cutaneous manifestations of gastrointestinal disease: part I. *Journal of the American Academy of Dermatology,* 68**,** 189 e1-21; quiz 210.

SHARAN, S. K., MORIMATSU, M., ALBRECHT, U., LIM, D. S., REGEL, E., DINH, C., SANDS, A., EICHELE, G., HASTY, P. & BRADLEY, A. 1997. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature,* 386**,** 804-10.

SHELDON, C. D., HODSON, M. E., CARPENTER, L. M. & SWERDLOW, A. J. 1993. A cohort study of cystic fibrosis and malignancy. *British journal of cancer,* 68**,** 1025-8.

SHEN, H., SPITZ, M. R., QIAO, Y., GUO, Z., WANG, L. E., BOSKEN, C. H., AMOS, C. I. & WEI, Q. 2003. Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. *Int J Cancer,* 107**,** 84-8.

SHEN, H., XU, Y., QIAN, Y., YU, R., QIN, Y., ZHOU, L., WANG, X., SPITZ, M. R. & WEI, Q. 2000. Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. *Int J Cancer,* 88**,** 601-6.

SHINMURA, K. & YOKOTA, J. 2001. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. *Antioxid Redox Signal,* 3**,** 597-609.

SHRIKHANDE, S. V., BARRETO, S. G., GOEL, M. & ARYA, S. 2012. Multimodality imaging of pancreatic ductal adenocarcinoma: a review of the literature. *HPB : the official journal of the International Hepato Pancreato Biliary Association,* 14**,** 658-68.

SILVERMAN, D. T., DUNN, J. A., HOOVER, R. N., SCHIFFMAN, M., LILLEMOE, K. D., SCHOENBERG, J. B., BROWN, L. M., GREENBERG, R. S., HAYES, R. B., SWANSON, G. M. & ET AL. 1994. Cigarette smoking and pancreas

cancer: a case-control study based on direct interviews. *J Natl Cancer Inst,* 86**,** 1510-6.

SILVERMAN, D. T., SCHIFFMAN, M., EVERHART, J., GOLDSTEIN, A., LILLEMOE, K. D., SWANSON, G. M., SCHWARTZ, A. G., BROWN, L. M., GREENBERG, R. S., SCHOENBERG, J. B., POTTERN, L. M., HOOVER, R. N. & FRAUMENI, J. F., JR. 1999. Diabetes mellitus, other medical conditions and familial history of cancer as risk factors for pancreatic cancer. *Br J Cancer,* 80**,** 1830-7.

SINGH, M. & MAITRA, A. 2007. Precursor lesions of pancreatic cancer: molecular pathology and clinical implications. *Pancreatology,* 7**,** 9-19.

SLATER, E., AMRILLAEVA, V., FENDRICH, V., BARTSCH, D., EARL, J., VITONE, L. J., NEOPTOLEMOS, J. P. & GREENHALF, W. 2007. Palladin mutation causes familial pancreatic cancer: absence in European families. *PLoS Med,* 4**,** e164.

SLIDELL, M. B., CHANG, D. C., CAMERON, J. L., WOLFGANG, C., HERMAN, J. M., SCHULICK, R. D., CHOTI, M. A. & PAWLIK, T. M. 2008. Impact of total lymph node count and lymph node ratio on staging and survival after pancreatectomy for pancreatic adenocarcinoma: a large, population-based analysis. *Annals of surgical oncology,* 15**,** 165-74.

SOBIN, L. H., GOSPODAROWICZ, M. K. & WITTEKIND, C. 2009. *TNM Classification of malignant tumours*.

SOHN, T. A., YEO, C. J., CAMERON, J. L., HRUBAN, R. H., FUKUSHIMA, N., CAMPBELL, K. A. & LILLEMOE, K. D. 2004. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. *Ann Surg,* 239**,** 788-97; discussion 797-9.

STELLMAN, S. D., CHEN, Y., MUSCAT, J. E., DJORDJEVIC, M. V., RICHIE, J. P., JR., LAZARUS, P., THOMPSON, S., ALTORKI, N., BERWICK, M., CITRON, M. L., HARLAP, S., KAUR, T. B., NEUGUT, A. I., OLSON, S., TRAVALINE, J. M., WITORSCH, P. & ZHANG, Z. F. 2003. Lung cancer risk in white and black Americans. *Annals of epidemiology,* 13**,** 294-302.

STOJADINOVIC, A., BROOKS, A., HOOS, A., JAQUES, D. P., CONLON, K. C. & BRENNAN, M. F. 2003. An evidence-based approach to the surgical management of resectable pancreatic adenocarcinoma. *J Am Coll Surg,* 196**,** 954-64.

SU, G. H., HRUBAN, R. H., BANSAL, R. K., BOVA, G. S., TANG, D. J., SHEKHER, M. C., WESTERMAN, A. M., ENTIUS, M. M., GOGGINS, M., YEO, C. J. & KERN, S. E. 1999. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. *Am J Pathol,* 154**,** 1835-40.

SUGIMURA, H., KOHNO, T., WAKAI, K., NAGURA, K., GENKA, K., IGARASHI, H., MORRIS, B. J., BABA, S., OHNO, Y., GAO, C., LI, Z., WANG, J., TAKEZAKI, T., TAJIMA, K., VARGA, T., SAWAGUCHI, T., LUM, J. K., MARTINSON, J. J., TSUGANE, S., IWAMASA, T., SHINMURA, K. & YOKOTA, J. 1999. hOGG1 Ser326Cys polymorphism and lung cancer susceptibility. *Cancer Epidemiol Biomarkers Prev,* 8**,** 669-74.

SULTANA, A., TUDUR SMITH, C., CUNNINGHAM, D., STARLING, N., TAIT, D., NEOPTOLEMOS, J. P. & GHANEH, P. 2007. Systematic review, including meta-analyses, on the management of locally advanced pancreatic cancer using radiation/combined modality therapy. *Br J Cancer,* 96**,** 1183-90.

SUNAGA, N., KOHNO, T., YANAGITANI, N., SUGIMURA, H., KUNITOH, H., TAMURA, T., TAKEI, Y., TSUCHIYA, S., SAITO, R. & YOKOTA, J. 2002. Contribution of the NQO1 and GSTT1 polymorphisms to lung adenocarcinoma susceptibility. *Cancer Epidemiol Biomarkers Prev,* 11**,** 730-8.

TADA, M., OHASHI, M., SHIRATORI, Y., OKUDAIRA, T., KOMATSU, Y., KAWABE, T., YOSHIDA, H., MACHINAMI, R., KISHI, K. & OMATA, M. 1996. Analysis of K-ras gene mutation in hyperplastic duct cells of the pancreas without pancreatic disease. *Gastroenterology,* 110**,** 227-31.

TAKEZAKI, T., GAO, C. M., WU, J. Z., LI, Z. Y., WANG, J. D., DING, J. H., LIU, Y. T., HU, X., XU, T. L., TAJIMA, K. & SUGIMURA, H. 2002. hOGG1 Ser(326)Cys polymorphism and modification by environmental factors of stomach cancer risk in Chinese. *Int J Cancer,* 99**,** 624-7.

TANAKA, M., CHARI, S., ADSAY, V., FERNANDEZ-DEL CASTILLO, C., FALCONI, M., SHIMIZU, M., YAMAGUCHI, K., YAMAO, K. & MATSUNO, S. 2006. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatology,* 6**,** 17-32.

TAYLOR, E. M. & LEHMANN, A. R. 1998. Conservation of eukaryotic DNA repair mechanisms. *International journal of radiation biology,* 74**,** 277-86.

TERRY, P. D., UMBACH, D. M. & TAYLOR, J. A. 2006. APE1 genotype and risk of bladder cancer: Evidence for effect modification by smoking. *Int J Cancer,* 118**,** 3170-3.

TERSMETTE, A. C., PETERSEN, G. M., OFFERHAUS, G. J., FALATKO, F. C., BRUNE, K. A., GOGGINS, M., ROZENBLUM, E., WILENTZ, R. E., YEO, C. J., CAMERON, J. L., KERN, S. E. & HRUBAN, R. H. 2001. Increased risk of incident pancreatic cancer among first-degree relatives of patients with familial pancreatic cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research,* 7**,** 738-44.

THOMPSON, L. H., BROOKMAN, K. W., JONES, N. J., ALLEN, S. A. & CARRANO, A. V. 1990. Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol Cell Biol,* 10**,** 6160-71.

THOMPSON, L. H. & WEST, M. G. 2000. XRCC1 keeps DNA from getting stranded. *Mutat Res,* 459**,** 1-18.

THRASH, B., PATEL, M., SHAH, K. R., BOLAND, C. R. & MENTER, A. 2013. Cutaneous manifestations of gastrointestinal disease: part II. *Journal of the American Academy of Dermatology,* 68**,** 211 e1-33; quiz 244-6.

TOFT, N. J. & ARENDS, M. J. 1998. DNA mismatch repair and colorectal cancer. *J Pathol,* 185**,** 123-9.

TOL, J. A., BUSCH, O. R., VAN DER GAAG, N. A., VAN GULIK, T. M. & GOUMA, D. J. 2012. The quandary of preresection biliary drainage for pancreatic cancer. *Cancer journal,* 18**,** 550-4.

TOMINAGA, S. 1999. Major avoidable risk factors of cancer. *Cancer Lett,* 143 Suppl 1**,** S19-23.

TOMIOKA, T., ANDREN-SANDBERG, A., FUJII, H., EGAMI, H., TAKIYAMA, Y. & POUR, P. M. 1990. Comparative histopathological findings in the pancreas of cigarette smokers and non-smokers. *Cancer Lett,* 55**,** 121-8.

TRAMACERE, I., SCOTTI, L., JENAB, M., BAGNARDI, V., BELLOCCO, R., ROTA, M., CORRAO, G., BRAVI, F., BOFFETTA, P. & LA VECCHIA, C. 2010. Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. *International journal of cancer. Journal international du cancer,* 126**,** 1474-86.

TSENG, J. F., TAMM, E. P., LEE, J. E., PISTERS, P. W. & EVANS, D. B. 2006. Venous resection in pancreatic cancer surgery. *Best Pract Res Clin Gastroenterol,* 20**,** 349-64.

TUIMALA, J., SZEKELY, G., GUNDY, S., HIRVONEN, A. & NORPPA, H. 2002. Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. *Carcinogenesis,* 23**,** 1003-8.

TURRINI, O., EWALD, J., BARBIER, L., MOKART, D., BLACHE, J. L. & DELPERO, J. R. 2013. Should the portal vein be routinely resected during pancreaticoduodenectomy for adenocarcinoma? *Annals of surgery,* 257**,** 726-30.

VAN ASPEREN, C. J., BROHET, R. M., MEIJERS-HEIJBOER, E. J., HOOGERBRUGGE, N., VERHOEF, S., VASEN, H. F., AUSEMS, M. G., MENKO, F. H., GOMEZ GARCIA, E. B., KLIJN, J. G., HOGERVORST, F. B., VAN HOUWELINGEN, J. C., VAN'T VEER, L. J., ROOKUS, M. A. & VAN LEEUWEN, F. E. 2005. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet,* 42**,** 711-9.

VAN DER GAAG, N. A., RAUWS, E. A., VAN EIJCK, C. H., BRUNO, M. J., VAN DER HARST, E., KUBBEN, F. J., GERRITSEN, J. J., GREVE, J. W., GERHARDS, M. F., DE HINGH, I. H., KLINKENBIJL, J. H., NIO, C. Y., DE CASTRO, S. M., BUSCH, O. R., VAN GULIK, T. M., BOSSUYT, P. M. & GOUMA, D. J. 2010. Preoperative biliary drainage for cancer of the head of the pancreas. *The New England journal of medicine,* 362**,** 129-37.

VAN ZEELAND, A. A., DE GROOT, A. J., HALL, J. & DONATO, F. 1999. 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutat Res,* 439**,** 249-57.

VARADHACHARY, G. R., WOLFF, R. A., CRANE, C. H., SUN, C. C., LEE, J. E., PISTERS, P. W., VAUTHEY, J. N., ABDALLA, E., WANG, H., STAERKEL, G. A., LEE, J. H., ROSS, W. A., TAMM, E. P., BHOSALE, P. R., KRISHNAN, S., DAS, P., HO, L., XIONG, H., ABBRUZZESE, J. L. & EVANS, D. B. 2008. Preoperative gemcitabine and cisplatin followed by gemcitabine-based chemoradiation for resectable adenocarcinoma of the pancreatic head. *J Clin Oncol,* 26**,** 3487-95.

VASEN, H. F., GRUIS, N. A., FRANTS, R. R., VAN DER VELDEN, P. A., HILLE, E. T. & BERGMAN, W. 2000. Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). *Int J Cancer,* 87**,** 809-11.

VIMALACHANDRAN, D., GHANEH, P., COSTELLO, E. & NEOPTOLEMOS, J. P. 2004. Genetics and prevention of pancreatic cancer. *Cancer Control,* 11**,** 6-14.

VOGEL, U., NEXO, B. A., OLSEN, A., THOMSEN, B., JACOBSEN, N. R., WALLIN, H., OVERVAD, K. & TJONNELAND, A. 2003. No association between OGG1 Ser326Cys polymorphism and breast cancer risk. *Cancer Epidemiol Biomarkers Prev,* 12**,** 170-1.

VOGEL, U., OLSEN, A., WALLIN, H., OVERVAD, K., TJONNELAND, A. & NEXO, B. A. 2004. No association between OGG1 Ser326Cys and risk of basal cell carcinoma. *Cancer Epidemiol Biomarkers Prev,* 13**,** 1680-1.

VOLKAN ADSAY, N. 2007. Cystic lesions of the pancreas. *Mod Pathol,* 20 Suppl 1**,** S71-93.

WAKATSUKI, T., IRISAWA, A., BHUTANI, M. S., HIKICHI, T., SHIBUKAWA, G., TAKAGI, T., YAMAMOTO, G., TAKAHASHI, Y., YAMADA, Y., WATANABE, K., OBARA, K., SUZUKI, T. & SATO, Y. 2005. Comparative study of diagnostic value of cytologic sampling by endoscopic ultrasonography-guided fine-needle aspiration and that by endoscopic retrograde pancreatography for the management of pancreatic mass without biliary stricture. *J Gastroenterol Hepatol,* 20**,** 1707-11.

WANG, B., WANG, D., HUANG, G., ZHANG, C., XU, D. H. & ZHOU, W. 2010. XRCC1 polymorphisms and risk of colorectal cancer: a meta-analysis. *International journal of colorectal disease,* 25**,** 313-21.
WANG, L., LIN, D. X., X.H., L., MIAO, X. P. & LI, H. 2006a. Polymorphisms of the DNA repair genes XRCC1 and XPC: relationship to pancreatic cancer risk. *Wei Sheng Yan Liu,* 35**,** 534-6.
WANG, Q., GURUSAMY, K. S., LIN, H., XIE, X. & WANG, C. 2008. Preoperative biliary drainage for obstructive jaundice. *Cochrane database of systematic reviews***,** CD005444.
WANG, S. S., DAVIS, S., CERHAN, J. R., HARTGE, P., SEVERSON, R. K., COZEN, W., LAN, Q., WELCH, R., CHANOCK, S. J. & ROTHMAN, N. 2006b. Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. *Carcinogenesis*.
WANG, Y., SPITZ, M. R., ZHU, Y., DONG, Q., SHETE, S. & WU, X. 2003. From genotype to phenotype: correlating XRCC1 polymorphisms with mutagen sensitivity. *DNA Repair (Amst),* 2**,** 901-8.
WARSHAW, A. L. & RUTLEDGE, P. L. 1987. Cystic tumors mistaken for pancreatic pseudocysts. *Ann Surg,* 205**,** 393-8.
WEIDERPASS, E., PARTANEN, T., KAAKS, R., VAINIO, H., PORTA, M., KAUPPINEN, T., OJAJARVI, A., BOFFETTA, P. & MALATS, N. 1998. Occurrence, trends and environment etiology of pancreatic cancer. *Scand J Work Environ Health,* 24**,** 165-74.
WEISS, J. M., GOODE, E. L., LADIGES, W. C. & ULRICH, C. M. 2005a. Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. *Mol Carcinog,* 42**,** 127-41.
WEISS, J. M., GOODE, E. L., LADIGES, W. C. & ULRICH, C. M. 2005b. Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. *Molecular carcinogenesis,* 42**,** 127-41.
WENG, H., WENG, Z., LU, Y., NAKAYAMA, K. & MORIMOTO, K. 2009. Effects of cigarette smoking, XRCC1 genetic polymorphisms, and age on basal DNA damage in human blood mononuclear cells. *Mutat Res,* 679**,** 59-64.
WHITCOMB, D. C., APPLEBAUM, S. & MARTIN, S. P. 1999. Hereditary pancreatitis and pancreatic carcinoma. *Ann N Y Acad Sci,* 880**,** 201-9.
WHO. 2010. *Genes and human disease* [Online]. WHO. [Accessed 01-02-2013.
WIENCKE, J. K. 2002. DNA adduct burden and tobacco carcinogenesis. *Oncogene,* 21**,** 7376-91.
WIGMORE, S. J., PLESTER, C. E., RICHARDSON, R. A. & FEARON, K. C. 1997a. Changes in nutritional status associated with unresectable pancreatic cancer. *British journal of cancer,* 75**,** 106-9.
WIGMORE, S. J., PLESTER, C. E., ROSS, J. A. & FEARON, K. C. 1997b. Contribution of anorexia and hypermetabolism to weight loss in anicteric patients with pancreatic cancer. *The British journal of surgery,* 84**,** 196-7.
WIKMAN, H., RISCH, A., KLIMEK, F., SCHMEZER, P., SPIEGELHALDER, B., DIENEMANN, H., KAYSER, K., SCHULZ, V., DRINGS, P. & BARTSCH, H. 2000. hOGG1 polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a caucasian population. *Int J Cancer,* 88**,** 932-7.
WILENTZ, R. E., GERADTS, J., MAYNARD, R., OFFERHAUS, G. J., KANG, M., GOGGINS, M., YEO, C. J., KERN, S. E. & HRUBAN, R. H. 1998. Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. *Cancer Res,* 58**,** 4740-4.
WILENTZ, R. E., GOGGINS, M., REDSTON, M., MARCUS, V. A., ADSAY, N. V., SOHN, T. A., KADKOL, S. S., YEO, C. J., CHOTI, M., ZAHURAK, M.,

JOHNSON, K., TASCILAR, M., OFFERHAUS, G. J., HRUBAN, R. H. & KERN, S. E. 2000a. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: A newly described and characterized entity. *Am J Pathol,* 156**,** 1641-51.

WILENTZ, R. E., IACOBUZIO-DONAHUE, C. A., ARGANI, P., MCCARTHY, D. M., PARSONS, J. L., YEO, C. J., KERN, S. E. & HRUBAN, R. H. 2000b. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res,* 60**,** 2002-6.

WOLFF, H. 2003. [Evolution of the treatment of pancreatic cancer]. *Zentralbl Chir,* 128**,** 443-7.

WOLPIN, B. M., KRAFT, P., GROSS, M., HELZLSOUER, K., BUENO-DE-MESQUITA, H. B., STEPLOWSKI, E., STOLZENBERG-SOLOMON, R. Z., ARSLAN, A. A., JACOBS, E. J., LACROIX, A., PETERSEN, G., ZHENG, W., ALBANES, D., ALLEN, N. E., AMUNDADOTTIR, L., ANDERSON, G., BOUTRON-RUAULT, M. C., BURING, J. E., CANZIAN, F., CHANOCK, S. J., CLIPP, S., GAZIANO, J. M., GIOVANNUCCI, E. L., HALLMANS, G., HANKINSON, S. E., HOOVER, R. N., HUNTER, D. J., HUTCHINSON, A., JACOBS, K., KOOPERBERG, C., LYNCH, S. M., MENDELSOHN, J. B., MICHAUD, D. S., OVERVAD, K., PATEL, A. V., RAJKOVIC, A., SANCHEZ, M. J., SHU, X. O., SLIMANI, N., THOMAS, G., TOBIAS, G. S., TRICHOPOULOS, D., VINEIS, P., VIRTAMO, J., WACTAWSKI-WENDE, J., YU, K., ZELENIUCH-JACQUOTTE, A., HARTGE, P. & FUCHS, C. S. 2010. Pancreatic cancer risk and ABO blood group alleles: results from the pancreatic cancer cohort consortium. *Cancer research,* 70**,** 1015-23.

WOOD, R. D. 1997. Nucleotide excision repair in mammalian cells. *The Journal of biological chemistry,* 272**,** 23465-8.

WU, X., ZHAO, H., SUK, R. & CHRISTIANI, D. C. 2004. Genetic susceptibility to tobacco-related cancer. *Oncogene,* 23**,** 6500-23.

XING, D. Y., TAN, W., SONG, N. & LIN, D. X. 2001. Ser326Cys polymorphism in hOGG1 gene and risk of esophageal cancer in a Chinese population. *Int J Cancer,* 95**,** 140-3.

XU, J., ZHENG, S. L., TURNER, A., ISAACS, S. D., WILEY, K. E., HAWKINS, G. A., CHANG, B. L., BLEECKER, E. R., WALSH, P. C., MEYERS, D. A. & ISAACS, W. B. 2002. Associations between hOGG1 sequence variants and prostate cancer susceptibility. *Cancer Res,* 62**,** 2253-7.

XUE, H., NI, P., LIN, B., XU, H. & HUANG, G. 2011. X-ray repair cross-complementing group 1 (XRCC1) genetic polymorphisms and gastric cancer risk: A HuGE review and meta-analysis. *American journal of epidemiology,* 173**,** 363-75.

YAMAMOTO, H., ITOH, F., NAKAMURA, H., FUKUSHIMA, H., SASAKI, S., PERUCHO, M. & IMAI, K. 2001. Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. *Cancer Res,* 61**,** 3139-44.

YEE, N. S., FURTH, E. E. & PACK, M. 2003. Clinicopathologic and molecular features of pancreatic adenocarcinoma associated with Peutz-Jeghers syndrome. *Cancer Biol Ther,* 2**,** 38-47.

YEO, C. J., ABRAMS, R. A., GROCHOW, L. B., SOHN, T. A., ORD, S. E., HRUBAN, R. H., ZAHURAK, M. L., DOOLEY, W. C., COLEMAN, J., SAUTER, P. K., PITT, H. A., LILLEMOE, K. D. & CAMERON, J. L. 1997. Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative

adjuvant chemoradiation improves survival. A prospective, single-institution experience. *Ann Surg,* 225**,** 621-33; discussion 633-6.
YEO, C. J. & CAMERON, J. L. 1999. Pancreatic cancer. *Curr Probl Surg,* 36**,** 59-152.
YEO, C. J., CAMERON, J. L., LILLEMOE, K. D., SOHN, T. A., CAMPBELL, K. A., SAUTER, P. K., COLEMAN, J., ABRAMS, R. A. & HRUBAN, R. H. 2002. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma, part 2: randomized controlled trial evaluating survival, morbidity, and mortality. *Annals of surgery,* 236**,** 355-66; discussion 366-8.
YEO, T. P., HRUBAN, R. H., BRODY, J., BRUNE, K., FITZGERALD, S. & YEO, C. J. 2009. Assessment of "gene-environment" interaction in cases of familial and sporadic pancreatic cancer. *J Gastrointest Surg,* 13**,** 1487-94.
YU, G., ZHU, M. H., ZHU, Z., NI, C. R., ZHENG, J. M. & LI, F. M. 2004a. Expression of ATM protein and its relationship with p53 in pancreatic carcinoma with tissue array. *Pancreas,* 28**,** 421-6.
YU, H. P., ZHANG, X. Y., WANG, X. L., SHI, L. Y., LI, Y. Y., LI, F., SU, Y. H., WANG, Y. J., LU, B., SUN, X., LU, W. H. & XU, S. Q. 2004b. DNA repair gene XRCC1 polymorphisms, smoking, and esophageal cancer risk. *Cancer Detect Prev,* 28**,** 194-9.
ZHANG, S., WANG, M., VILLALTA, P. W., LINDGREN, B. R., UPADHYAYA, P., LAO, Y. & HECHT, S. S. 2009. Analysis of pyridyloxobutyl and pyridylhydroxybutyl DNA adducts in extrahepatic tissues of F344 rats treated chronically with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Chem Res Toxicol,* 22**,** 926-36.
ZHANG, X. M., MITCHELL, D. G., DOHKE, M., HOLLAND, G. A. & PARKER, L. 2002. Pancreatic cysts: depiction on single-shot fast spin-echo MR images. *Radiology,* 223**,** 547-53.
ZHANG, Y., NEWCOMB, P. A., EGAN, K. M., TITUS-ERNSTOFF, L., CHANOCK, S., WELCH, R., BRINTON, L. A., LISSOWSKA, J., BARDIN-MIKOLAJCZAK, A., PEPLONSKA, B., SZESZENIA-DABROWSKA, N., ZATONSKI, W. & GARCIA-CLOSAS, M. 2006. Genetic polymorphisms in base-excision repair pathway genes and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev,* 15**,** 353-8.
ZHANG, Z., SHI, Q., WANG, L. E., STURGIS, E. M., SPITZ, M. R., EL-NAGGAR, A. K., HONG, W. K. & WEI, Q. 2004. No Association between hOGG1 Ser326Cys polymorphism and risk of squamous cell carcinoma of the head and neck. *Cancer Epidemiol Biomarkers Prev,* 13**,** 1081-3.
ZIENOLDDINY, S., CAMPA, D., LIND, H., RYBERG, D., SKAUG, V., STANGELAND, L., PHILLIPS, D. H., CANZIAN, F. & HAUGEN, A. 2006. Polymorphisms of DNA repair genes and risk of non-small cell lung cancer. *Carcinogenesis,* 27**,** 560-567.
ZIOGAS, A. & ANTON-CULVER, H. 2003. Validation of family history data in cancer family registries. *Am J Prev Med,* 24**,** 190-8.

Publications

Oral presentation

Decreased tobacco exposure in patients with pancreatic ductal adenocarcinoma who have a family history of cancer in first degree relatives.

7th World Congress of the International Hepato-Pancreato-Biliary Association in Edinburgh 3-7 September 2006

Oral presentation in the BJS Prize Session

Abstracts

Decreased tobacco exposure in patients with pancreatic ductal adenocarcinoma who have a family history of cancer in first degree relatives

HPB Sept 2006;vol8, Supplement 2: Page 23

Publications

1. The role of genetic susceptibility in sporadic pancreatic ductal adenocarcinoma

   R Lochan, AK Daly, HL Reeves, RM Charnley

   British Journal of Surgery 2008 Jan;95(1):22-32 PMID: 18076020

2. Family history of cancer and tobacco exposure in index cases of pancreatic ductal adencarcinoma

   Lochan R, Daly AK, Reeves HL, Charnley RM

   J Oncol. 2011;2011:215985. Epub 2011 Apr 6 PMID: 21547248

3. Cyclooxygenase-2 Polymorphisms and Pancreatic Cancer Susceptibility

   Ozhan G, Lochan R, Leathart JB, Charnley R, Daly AK.

   Pancreas. 2011 Jun 23. [Epub ahead of print] PMID: 21705955

4. The role of tobacco derived carcinogens in pancreatic cancer

   Lochan R, Daly AK, Reeves HL, Charnley RM

   ISRN Oncology (In press)

Appendices

Appendix 1: Studies of hOGG1 polymorphisms and risk of malignancy

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases number	Controls number	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	Hardy-Weinberg p value	Ref
Lung	Sugimura	1999	Japan	Asian	241	197	Hospital	No	54.3	13.7	0.08	(Sugimura et al., 1999)
	Ito	2002	Japan	Asian	138	240	Hospital	No info	49.2	22.5	0.84	(Ito et al., 2002)
	Sunaga	2002	Japan	Asian	198	152	Hospital	No	43.4	23.7	0.13	(Sunaga et al., 2002)
	Lan	2004	China	Asian	118	109	Population	Frequency	39.4	13.8	0.23	(Klein et al., 2004)
	Le Marchand	2002	Hawaii	Japanese, Caucasian, Hawaiian	298	405	Population	Frequency	43.2	13.1	0.35	(Le Marchand et al., 2002)
	Park	2004	United States	Caucasian, Other unspecified ethnic	179	350	Screening	Individual	24.9	2.3	0.86	(Park et al., 2004)

				groups								
	Wikman	2000	Germany	Caucasian	105	105	Hospital	Frequency	41.0	1.9	0.07	(Wikman et al., 2000)
	Hung	2005	Europe	Caucasian, Other unspecified ethnic groups	2155	2163	Hospital	Frequency	33.1	3.7	0.22	(Hung et al., 2005a)

OGG1 studies (contd)

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases	Controls	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	H W	Ref
Oeso Ca	Xing	2001	China	Asian	196	201	Hospital Healthy	Frequency	52.7	13.4	0.15	(Xing et al., 2001)
NPC	Cho	2003	Taiwan	Asian	333	283	Community	Frequency	45.6	38.2	0.48	(Cho et al., 2003)
Oeso Ca	Hao	2004	China	Asian	419	480	Population	Frequency	45.0	16.5	0.24	(Hao et al., 2004)
Orolaryn Ca	Elahi	2002	United States	Caucasian	167	331	Hospital healthy	Frequency	23.0	1.8	0.94	(Elahi et al., 2002)
H & N Sq Ca	Zhang	2004	United States	Caucasian, UEG	706	1196	Hospiatl healthy	Frequency	32.4	5.8	0.06	(Zhang et al., 2004)
Colon	Kim	2003	Korea	Asian	125	247	Hospiatl healthy	Frequency	53.0	25.9	0.32	
Stomach	Takezaki	2002	China	Asian	101	198	Population	Frequency	60.6	24.2	<0.01	(Takezaki et

												al., 2002)
Stomach	Hanoaka	2001	Brazil	Asian	58	127	Hospital	Hospiatl	44.1	21.3	0.25	(Hanaoka et al., 2001)
Stomach	Hanoaka	2001	Brazil	Non-Japanese Brazilian	208	205	Hospital	Individual	36.1	3.9	0.44	(Hanaoka et al., 2001)
Sporadic Prostate cancer	Xu	2002	United States	Caucasian	199	174	Hospital healthy	No	36.2	8.6	0.32	(Xu et al., 2002)

OGG1 studies (contd)

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases	Controls	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	H W	Ref
Hereditary prostate	Xu	2002	United States	Caucasian	99	174	Hospital healthy	No	36.2	8.6	0.32	(Xu et al.,

cancer												2002)
Basal cell carcinoma	Vogel	2004	Denmark	Caucasian	319	319	Population	Individual	39.2	8.5	0.60	(Vogel et al., 2004)
Breast	Vogel	2003	Denmark	Caucasian	425	434	Population	Individual	38.9	4.6	0.18	(Vogel et al., 2003)
Breast	Choi	2003	Korea, Japan	Asian	466	466	Hospital	No	52.1	24.1	0.36	(Choi et al., 2003)
NSCLC	Zienolddiny	2006	Norway	Caucasian	343	413	Population					(Zienolddiny et al., 2006)
Breast	Zhang	2006	United States									(Zhang et al., 2006)
Breast	Zhang	2006	Poland									(Zhang et al., 2006)
NHL	Wang	2006			1172	982	Population					(Wang et al., 2006b)

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases	Controls	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	H W	Ref
Hereditary prostate cancer	Xu	2002	United States	Caucasian	99	174	Hospital healthy	No	36.2	8.6	0.32	(Xu et al., 2002)
Basal cell carcinoma	Vogel	2004	Denmark	Caucasian	319	319	Population	Individual	39.2	8.5	0.60	(Vogel et al., 2004)
Breast	Vogel	2003	Denmark	Caucasian	425	434	Population	Individual	38.9	4.6	0.18	(Vogel et al., 2003)
Breast	Choi	2003	Korea, Japan	Asian	466	466	Hospital	No	52.1	24.1	0.36	(Choi et al., 2003)
NSCLC	Zienolddiny	2006	Norway	Caucasian	343	413	Population					(Zienolddiny et al., 2006)
Breast	Zhang	2006	United States									(Zhang et al., 2006)
Breast	Zhang	2006	Poland									(Zhang et al.,

												2006)
NHL	Wang	2006			1172	982	Population					(Wang et al., 2006b)

Appendix 2: Studies of APE1/APEX1 polymorphisms and risk of malignancy

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases	Controls	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	H W	Ref
Lung	Ito	2004	Japan	Asian	178	449	Hospital	Frequency	50.3	14.3	0.25	(Ito et al., 2004)
	Misra	2004	Finland	Caucasian	310	302	Population	Individual	53.0	25.5	0.29	(Misra et al., 2003)
	Popanda	2004	Germany	Caucasian	459	457	Hospital	No	51.0	23.2	0.66	(Popanda et al., 2004)
UGI tract	Hao	2004	China	Asian	409	478	Population	Frequency	49.0	19.9	0.86	(Hao et al., 2004)

Appendix 3: Selected studies of XRCC1 Arg194Trp polymorphisms and risk of malignancy

Cancer type/site	First author	Year	Country	Ethnicity of subjects	Cases	Controls	Source of controls	Matching	Heterozygotes	Rare allele Homozygote	H W	Ref
Lung	Ratnasinghe	2001	China	Asian	108	216	Population	Individual	48.1	9.7	0.26	
	Chen	2002	China	Asian	109	109	Population	Individual	36.1	4.6	0.69	
	Hung	2005	Europe	Caucasian	2147	2132	Hospital	Frequency	13.7	0.6	0.93	
UGI Tract	Lee	2001	Taiwan	Asian	105	264	Hospital	Frequency	45.5	7.2	0.17	
	Xing	2002	China	Asian	433	524	Population	Frequency	43.5	7.1	0.16	

Appendix 4: Tobacco smoking and pancreatic cancer

	Type of Study	Year/Study period (Incident cases)	Geographic population studied	Size of study population	Risk for Pancreatic Cancer	Note		Reference
1.	Population based Case-control study	1984-88	Central Netherlands	189 cases, 702 controls	1.96			Bueno De Mesquita 1991
2. 1	Multi-centre Case-control study. Population based	Varied acc to centre. 1983-88	Adelaide, Toronto, Utretcht, Opole, Montreal	832 cases, 1679 controls	Non-smokers 1.0 Ever smokers 1.91-2.70	SEARCH programme of IARC	Risk increased in a significant trend with increasing life-time exposure in all centres independently and collectively. Quitting for 15 years decreased risk to that of non-smokers	Boyle 1996
3.	Population based case-control study	1985-88	Opole, Poland	110 cases, 195 cases	OR ever smoker 1.89	Only 43 % cases were pathologically confirmed	Weak association betwn life-time consumption of cigs and risk	Zatonski 1993

4.	Hospital based case-control study	1982-85	Paris, France	161 cases, 268 controls	No increased risk for tobacco smoking and Panc Ca	Only 102 cases were histologically confirmed		Clavel 1988
	Type of Study	**Year/Study period (Incident cases)**	**Geographic population studied**	**Size of study population**	**Risk for Pancreatic Cancer**	**Note**		**Reference**
5.	Prospective Cohort study	National Health Screening Service Survey 1984-1986. 12 years follow-up	Nord-Trondelag, Norway	31000 men & 32374 women	RR current smokers 2.1 (men) 2.1 (women).		Clear statistical association between dose and response for both men & women	Nilsen 2000
6.	Cohort study	Nurse Health Study (1976). Health Professionals Follow-up Study (1986)	118339 women. 49428 men. US men and women.	2 large prospective cohorts – Nurses Health Study (1976) and Health professionals Follow-up Study(1986).	RR (overall) never smokers 1, Former smokers 1.2, current smokers 2.5. RR (women) NS	Every 2-years follow-up questionnaires were sent by mail to update info:.	No clear dose-response relationship betwn no: of cig's and RR in current smokers. Total proportion of pancreatic cancers	Fuchs 1996

					1, FS 1.1, CS 2.4. RR (men) NS 1, FS 1.3, CS 3.		attributable to smoking was 25%	
	Type of Study	**Year/Study period (Incident cases)**	**Geographic population studied**	**Size of study population**	**Risk for Pancreatic Cancer**	**Note**		**Reference**
	Prospective Cohort study	1968-95	Iceland	11580 females, 11366 males as a part of the Icelandic cardiovascular risk factor study	Men: RR former smoker 2.37, pipe 2.52, cigar 4.87, 1-14 cigs/day 7.18, 15-24 cigs/day 10.2, 25+/day 12.5	Patient filled in a mailed questionnaire and brought it in to the clinic.	Dose response relationship in men demonstrated. Smoking is a risk factor in females only when 25+ cig's/day (RR 4.52) but the 95% CI is very wide (1.02-20.1) and after adjustment for other risk factors the RR is not significant	Tulinius 1997

7.	Prospective cohort study	1986-94	Iowa. Iowa women's Health Study	33976 postmenopausal women from Iowa	Less than 20 pack years RR 1.14, more than 20 pack years 1.92 (p for trend 0.02)	Mailed questionnaire	Coffee consumption and alcohol consumption increased risk after adjustment for age and smoking. Dose response relationship demonstrated.	Harnack 1997
	Type of Study	**Year/Study period (Incident cases)**	**Geographic population studied**	**Size of study population**	**Risk for Pancreatic Cancer**	**Note**		**Reference**
8.	Case-control	1986-89	Atlanta, Detroit and Michigan. USA	526 cases, 2153 controls. Population based.	OR 1.1 – 2.2. Inc trend with no: cigs (p=0.001)	Direct interviews with subjects	Relationship between cig's smoking and panc Ca:. causal. Dose-response relationship demonstrated.	Silverman 1994

							Former smokers who stopped smoking had a 30% reduction in risk compared to non-smokers.	
9.	Cohort study	1963 cohort	Swedish men and women	27841 men and 28089 women. Smoking habit survey 1963.	RR inc from 2.35 to 6.44 with inc no: of cig's in men.	Smoking and risk of mortality from panc ca evaluated	Dose response relation ship in men. Few women smoking a very high no; of cig's/day.	Nilsson 2005
10.	Prospective cohort study	1982 cohort	U.S men	137243 men. American cancer Society's Cancer Prevention study 2 cohort	RR for "inhalers" 2.7 (95% CI 1.5-4.8)	Cigar smoking and risk of mortality evaluated	No increased risk for death from pancreas cancer fro non-inhalers	Shapiro 2000
	Type of Study	**Year/Study period (Incident cases)**	**Geographic population studied**	**Size of study population**	**Risk for Pancreatic Cancer**	**Note**		**Reference**
11.	Case-control	1991-92	Athens, Greece.	181 cases. 181 hospital patient controls and 181 hospital visitors	OR and RR not calculated		Increased risk for pancreatic cancer in smokers, but not quantified.	Kalapothaki 1993

				as controls			Suggestion of earlier age at menarche associated with inc risk and parous women at lower risk.	
12.	Case-control	1990-93	Shanghai, China	451 cases, controls 1552. Population based.	OR 1.4 – 5.0. OR higher in men than women. Inc trend with no: ($p<$ 0.0001 in men and p=0.05 in women)	Direct interviews	Dose-response relationship demonstrated. Formwe smokers who stopped smoking for more than 10 years had risks comparable to non-smokers.	Ji 1995
	Type of Study	**Year/Study period**	**Geographic population**	**Size of study population**	**Risk for**	**Note**		**Reference**

		(Incident cases)	**studied**		**Pancreatic Cancer**			
13.	Cohort study	1966-81	Japanese men & women	265000	Males 1.1 to 1.6 with inc in no: of cis's (p for trend 0.04). Females 1.4 to 1.9 (p for trend 0.02)	Re-analysis of the six prefecture cohort data	Smoking more than 34 cigs for men and more than 15 cigs/day in women did not inc risk but dec risk of rPanc Ca.m	Akiba 1990
14.	Cohort study	1965-93	Norwegian men & women	26,000	RR current smoker 1.3 (men) 1.4 (women)	Self-administered mailed questionnaire	A subsequent analysis (Heuch) of the same cohort showed an inc RR when only histologically confirmed cases were included in the analyses	Engeland 1996
15.	Cohort study	1941-75	Mortality data analysis England & Wales	All deaths due to panc. Ca betwn 1941-71.	RR of 1.6 for 20 pack years of smoking		Cohort analysis VERY COMPLICATED STATS!!!	Moolgavkar 1981

	Type of Study	**Year/Study period (Incident cases)**	**Geographic population studied**	**Size of study population**	**Risk for Pancreatic Cancer**	**Note**		**Reference**
16.	Longitudinal cohort study	1963-79	Swedish men	25129 men	Relative death rate for Curr cig smok 3.3, for curr pipe smoker 2.8 and curr cigar smok 1.0	Inc risk for death from panc ca in both cig and pipe smokers. Risk inc with increasing consumption of tobacco in curr smokers and for ex-smokers	No influence of age of commencement of smoking on risk of death from pancreatic cancer	Carstensen 1987
17.	Case-control	1976 – all cases under 65 years of age	Los Angeles county, North America	490 cases, age, sex matched controls	Current: 4.3-5.7 (RR)	Ex-smokers of > 10 yrs no increased risk.		Mack 1986
18.	Case-control	1985-93	New York,	484 cases,	OR upto 1.4 (30-39 cig	Pancreatic cancer is	All direct interviews with	Muscat

			North America	954 controls	yrs in males) 2.4 (20-29 cig yrs). Further increase in exposure did not increase OR	caused by smoking in women too AND the risks are higher than in men for the same amount and duration of tobacco consumption.	incident cases and controls (hospital derived). Males and females analysed separately.	1997
19.	Case-control	1984-88	Greater Montreal, Canada	179 cases, 239 controls	OR 3.76 for highest quintile (unfiltered cig 5.08)	Statistically significant increase in trend of OR with increase in number of cigs smoked	Population based controls	Ghadirian 1990
20.	Case-control	1983-88	Greater Milan, North Italy	214 cases, 1944 controls	Never RR 1 Ex RR 1.23 Current RR Less than 15: 0.75 15-24: 1.16 ≥ 25 1.44	Increasing exposure lead to increase in RR	Controls – subjects admitted to hospital with non-acute non-neoplastic GI disorders	Ferraroni etal 1989

21.	Prospective mortality study (cohort)	1982-86	North American women	700,000	RR not calculated. Attributable risk (current smokers) 55.2%	Attributable risk (former smokers) 42.4%. All subjects PAR% 25.6%		Stellman 1989
22.	Longitudinal study Cohort	1960-1989	Females in Sweden	26000	RR for former smoker [2.47(1.14 – 5.34)]. RR current smoker [1.77(1.09-2.87)]	Former smokers at a higher risk for panc Ca!!!	Elevated RR for former and current smokers compared with women who did not smoke regularly – cancers of lung, upper aero-digestive tract, pancreas, cervix, bladder.	Nordlund 1997
23.	Cohort study	1967 - 75	Mormons of Utah	900000 popln. for Utah. 19,940 deaths in the study period analysed	30 % - 40 % decrease in incidence of pancreatic cancer as compared to national average (from SEER, TNCS)	Mormons refrain from use of alcohol, tobacco, non-medicinal drugs and place emphasis on family values, strict sexual mores and	Mortality data compared between Mormons & Non-mormons in Utah.	Lyon 1980

						education		
24.	Cohort study?	1970-82	Male veterans utilizing Veterans Administration system betn 1970-82	37,04,000	No difference in age-specific incidence rates and curves for pancreatic cancer as compared to SEER registry	VA users exhibit a very high proportion of blacks and prevalence of chronic cigarette (nearly double) smoking, alcohol use and poor nutrition	The absence of an increase in panc Ca (as opposed to the nearly double the incidence of upper aero-digestive and Resp cancers) as compared to SEER data would be explained by the competing risks of other malignancies and illnessess	Harris etal 1989
25.	Correlation study	1972-76	5 ethnic groups in Hawai – Hawaian, Japanese, Chinese, Filipjno and Caucasians	Personal interviews of 9920 individuals	Significant risk of pancreatic cancer predicted for use of Tobacco. Predicted annual cancer	Using cancer incidence data and tobacco, alcohol consumption based on personal interview		Hinds etal. 1980

					incidence/ 100000 popln 10 PY: 19.2 20 PY: 28.9			
26.	Population based cross-sectional study					Lot of errors in analysis methods		Roger R Williams etal 1976
27.	Population based case-control study	1986-1989	Iowa	3574 cancer cases (376 pancreas cases). Controls 2434 [6.5 controls :1 case]	OR: males 1.8 95% CI 1.2-2.8; females 2.1 1.4-3.1	mailed questionnaire	Multi site CC study	c-h-chiu etal 2000
28.	Multi site case control study	1979 - 1985	Montreal	3730 cancer patients. 533 controls	OR: 1.6% PARP: 33%		Multi site CC study	Siemiatycki J et al. 1995
29.	Prospective mortality study	Enrolment 1982. F/U 4 years	Across all 50 states in America	1.2 million (men & women) enrolled & smoking history collected. Analysis performed on	Attributable risk of death due to pancreatic cancer for current smokers 55.2%		1527 deaths due to 6 smoking related cancer sites	Stellman 1989

				685,748 women	(former smokers 42.4)			

Appendix 5: MONICA tobacco smoking questionnaire

1. Do you smoke cigarettes now?
   1 = yes, regularly Go to 2. |__|
   2 = no Go to 5.
   3 = occasionally Go to 3.

2. On average, how many cigarettes do you smoke a day?
   Go to 7. Number: |__|__|__|

3. On how many days a week do you smoke cigarettes?
   1 = usually on one day or less |__|
   2 = usually on 2 to 4 days
   3 = almost every day

4. On average, how many cigarettes do you smoke a day?
   Number: |__|__|__|

5. Did you ever smoke cigarettes regularly in the past?
   1 = yes Go to 6. |__|
   2 = no Go to 9.

6. When did you stop smoking cigarettes regularly? Year, 19-- |__|__|
   If in the last 12 months
   1 = less than 1 month ago |__|
   2 = 1-6 months ago
   3 = 6-12 months ago

7. What is the highest average daily number of cigarettes you have ever smoked for as long as a year? Number: |__|__|__|

8. How old were you when you began to smoke cigarettes regularly? Age: |__|__|

9. Have you ever smoked cigars/cigarillos?
   1 = now smoke regularly Go to 10. |__|
   2 = no Go to 11.
   3 = now smoke occasionally (less than one/day) Go to 10.
   4 = used to, but not now Go to 11.

10. How many do you smoke per week? Number: |__|__|__|

11. Have you ever smoked a pipe?
   1 = now smoke regularly Go to 12. |__|
   2 = no Go to 13.
   3 = now smoke occasionally (less than once a day) Go to 12.

4 = used to, but not now Go to 13.

12. About how many grams of tobacco do you smoke per week? Grams: |__|__|__|

13. To be completed by occasional and non-smokers only (i.e. when item 1 is coded 2 or 3):
For how many hours, on average each day, are you closely subjected to other people's tobacco smoke? |__|__|