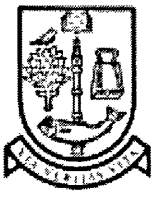

UNIVERSITY
of
GLASGOW

DISTRIBUTION OF HLA CLASS II AND MHC CLASS III ALLELES IN GASTRIC CANCER

A thesis presented by

NIKOLAOS LYRAKOS B.Sc. (Hons), AIBMS, GIBiol

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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This thesis is dedicated to

my parents, my two brothers and my grandfather

and also to the rest of all my family for their encouragement over the years

and to the

memory of my grandmother Mrs Aristea Manta

who died from cancer in 1993.

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DECLARATION

The work in this thesis is based on my own independent work except where acknowledged.

NIKOLAOS LYRAKOS BSc. (Hon), AIBMS, GIBiol

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LIST OF ABBREVIATIONS

The following abbreviations have been used throughout this thesis:

Ags:	Antigens
APC:	Adenomatus polyposis coli
APS:	Ammonium persulphate
BFGF-2:	Basic fibroblast growth factor-2
CagA:	Cytotoxin associated gene
CMM:	Cutaneous malignant melanoma
°C:	Degrees Celsius (centigrade)
DCC:	Deleted in colon carcinoma gene
EBV:	Ebsten-barr-virus
EDTA	Ethylenedianinetetra-acetic acid disodium salt
EGF:	Epidermal growth receptor
EGFR:	Epidermal growth factor receptor
FGF:	Fibroblast growth factor
g:	Gram
GF:	Growth factor
GORD:	Gastroesophagial reflux disease
HGF:	Hepatocyte growth factor
HLA:	Human leukocyte antigens
HNPCC:	Hereditary non-polyposis colon cancer syndrome genes
HPV:	Human papilloma virus
hr:	Hour
HSP:	Heat shock protein
ICAM-1:	Intercellular adhesion molecules
IDDM:	Insulin-dependent diabetes mellitus
IFN:	Interferon

Ig:	Immunoglobulin
IGF-II:	Insulin like growth factor-2
IL:	Interleukin
Kb:	Kilobases
LOH:	Lost of heterozygosity
M:	Molar
mA	Milliamperes
Mb:	Megabases
MCC:	Mutated in colon carcinoma gene
mg:	Milligram (grams × 10^{-3})
μg:	Microgram (grams × 10^{-6})
MHC:	Major histocompatibility complex
MIN:	Microsatellite instability
min:	Minute
ml:	Millilitre (10^{-3} L)
μl:	Microlitre
mM:	Millimolar (10^{-3} M)
μM	Micromolar (10^{-6} M)
mol:	Mole
mRNA:	Messenger RNA
MS:	Multiple sclerosis
MUC-1:	Mucin proteins
NF-kB:	Nuclear factor -kB
ng:	Nanograms (grams × 10^{-9})
NK:	Natural killer cells
PCR:	Polymerase chain reaction
PCR-SSP:	Polymerase chain reaction-sequence specific primer

PDGF:	Platelet derived growth factor
RA:	Rheumatoid arthritis
RO:	Reactive oxygen
SCCs:	Squamus cell carcinomas
Sec:	Second
STRs:	Simple tandem repeats
SLE:	Systemic lupus erythematosus
TBE:	Tris / borate / EDTA buffer
TEMED:	N, N, N", N-tetramethylethylenediamine
TGF:	Transforming growth factor
Th1:	T-helper lymphocytes
Tm	Temperature (oligonucleotide melting temperature)
TNF:	Tumour necrosis factor
VacA:	Vacuolating cytotoxin A
VCAM-1:	Vascular cell adhesion molecules
VNTRs:	Variable number tandem repeats

ABSTRACT

The progression from normal to neoplastic cells takes place over an undefined period under the combined influence of several factors, including genetic susceptibility. The MHC system (HLA in humans) is composed of genes that control a variety of immune functions and influence the susceptibility for more than 40 diseases, including cancer. This study was to investigate the relationship among cytokine related genetic loci on chromosome 6 that might be involved in Barrett's oesophagus and in gastric cancer that involves a major "environmental factor" *Helicobacter Pylori* infection. The genetic loci investigated were the HLA-DQB1 locus, the HSP-70-2 locus and D6S273 marker and compared with TNFa 1-14 microsatellites, on chromosome 6, in region 2, band 1, sub-band 3. The subjects taking part in this investigation were patients with Barrett's oesophagus, patients with gastric cancer and a cancer free population, all originated from the West of Scotland. The protocols used were PCR based and radioactive PCR microsatellite analysis, performed in order to refine the genetic contribution of the three loci that were investigated, in gastric cancer and in Barrett's oesophagus conditions and to fine map the relationship of the TNF locus with adjacent genetic loci that span from the HLA class-II region to HLA-B locus.

The results obtained suggest that there is a significant difference in genotype and allelotype distribution in certain loci. Significant associations were discovered in the DQβ1 locus for the DQ 3 serotype (gastric cancer Vs controls; P = 0.0421), in the HSP 70-2 locus genotype 1 * 2 (malignant Barrett's Vs controls; P = 0.0194), in the D6S273 locus genotype 132-134 (malignant Barrett's Vs controls; P = 0.0337) and the allelotype (serotype) DQ 6 (total Barrett's population Vs controls; P= 0.0252). Also there are significant differences in allelotype distribution among certain loci where significant association was discovered between the DQβ1 locus and the HSP 70-2 locus (DQ 2 - HSP (936bp) alleles in gastric population Vs controls; P= 0.0487). Additionally, there is not a defined difference in genotype-allelotype distribution and no significant association was discovered among the three loci tested compared with the TNFa 1-14 microsatellites.

Our study leads to the conclusion that the HLA-DQβ1, HSP-70-2 and D6S273 loci are associated with gastric cancer and Barrett's oesophagus and that the HLA-DQβ1 and the HSP-70-2 loci are in linkage with each other for the genotype (DQ 2 - HSP (936bp). In addition none of the three loci tested are in close linkage with the TNF locus.

Encouraging results were obtained, and there could be a possibility that new immunogenetic therapeutic procedures for Barrett's oesophagus and gastric cancer could be based on these specific immunogenetic targets (i.e. HLA-DQβ1, HSP-70-2, and D6S273), their protein products and relative cytokines.

INTRODUCTION

INTRODUCTION

1. CHARACTERISATION OF CANCER

Cells within our bodies have the ability to adapt in response to environmental changes. This is accomplished by altering their growth mechanism or their metabolism. A key feature of such alterations is that if the stimulus is removed, cells revert to their normal growth, function or metabolic activity. There are certain environmental stimuli that can cause drastic alterations to the genetic material of a cell, such as chemical substances or UV radiation that may result in permanent modification to cellular growth. This process is known as metaplasia. There are also many factors that may influence such modifications although a person's "lifestyle" could be one of the main influences (such as food, drink or tobacco consumption for example). If these stimuli are combined, uncontrolled division and cell invasion occurs, which is otherwise known as neoplasia. They proliferate excessively forming a lump or a tissue mass known as a neoplasm. A mass of neoplastic cells is commonly known as tumour, derived from the Latin "*tumere*" meaning to swell.

Despite all these generalisations, cancer is now known to be a multifactorial disease. The term "lifestyle" accommodates numerous factors that are known to be associated with susceptibility to the disease such as diet, smoking and exposure to synthetic and natural agents. Diseases that are derived from viruses, bacteria, mutations in suppressor genes and oncogenes are known also to play a crucial role in the development of cancer. Thus, an acceptable definition of cancer would be: a set of diseases, characterised by unregulated cell growth leading to invasion of surrounding tissues and metastasising to other parts of the body (Stevens & Lowe, 1995; King, 1996).

CLASSIFICATION OF NEOPLASMS

There are two main types of neoplastic growth. A tumour that is well defined and grows only locally is termed benign. In contrast, when the neoplastic cells are growing into and destroying the surrounding tissues the neoplasm is termed malignant. The main difference between benign and malignant tumours is that the malignant tumours are invasive and metastasising. Benign tumours have a much better prognosis than malignant. They normally grow very slowly, remain localised and do not show a highly abnormal deregulation. The main feature of such tumours is that they cause compression of adjacent tissues. Such abnormal growth and lack of full differentiation of benign neoplastic cells reflects the abnormal function and morphology of tissues or organs. Malignant neoplastic cells represent variable degrees of differentiation. When tumour cells present only a small resemblance to the original tissue, then the neoplasm is termed "a poorly-differentiated" malignant neoplasm. In the case of complete failure of differentiation, where it is not possible to identify the precursor cell, then the growth is termed "anaplastic" malignant neoplasm. Finally when the tumour cells resemble the original tissue then the tumour is termed "a well-differentiated" malignant neoplasm. Generally, poorly differentiated neoplasms tend to be more aggressive than well differentiated. Morphological variations such as size and shape of cells or nucleus are commonly seen in both types of neoplasms. This is known as atypical cytology (Stevens & Lowe, 1995; King, 1996).

INVASION AND METASTASIS

Invasion and metastasis are key features of malignant neoplasms. It is an inefficient process as most cells are destroyed in transportation to other parts of the body but efficient in the sense that most human cancers metastasise successfully. The control of cell growth in primary tumours becomes so abnormal that cells grow into adjacent tissues, a process known as invasion. Metastasis is the escape of cancer cells from primary sites and their localisation at secondary sites. Metastasising cells continue to grow abnormally

constructing a new tumour at the new site, known as a secondary tumour. Treatment of such malignant metaplastic conditions is very difficult due to the fact that systemic eradication is required. Metastasis requires a disruption of local cell – cell interactions, invasion, and penetration into blood or lymphatic vessels, escape from these vessels, migration and growth. The organ that the transporting vessels first pass through is usually the first organ in which the metastases occur. Adhesion molecules, endothelial function and extra cellular matrix composition all contribute to metastatic process. Individual metastasis can be of clonal origin. Invasion and migration requires proteolytic enzymes and polypeptide mobility factors. Arrest of cancer cells within vessels is possible as a result of passive entrapment from lymphocytes, platelets and endothelial cells. Growth of metaplastic tumours and primary tumours require the development of blood vessels this process is known as angiogenesis. Angiogenesis requires angiogenic factors, the most characteristic of which is heparin binding fibroblast growth factors (Stevens & Lowe, 1995; King, 1996).

CANCER AND GENE CHARACTERIZATION

Most of the fundamental ideas on causes of cancer were originated around the turn of last century. The distinguished British surgeon Percival Potts, 200 years ago identified the first occupational cause of cancer, among chimney sweeps due to their exposure to soot. Thus, the first type of cancer described to a specific cause was cancer of the scrotum. Later other causes were described such as abnormalities of differentiation, viruses, defects of the immune system, chromosomal abnormalities, such as that of the tpr-met (Soman *et al.*, 1991) gene translocation in gastric cancer. All these causes of cancer can now be combined into one main concept that cancer arises from a series of changes in the expression of genes. Today, evidence suggests that specific genetic changes occur in cancer cells from a clonal origin. Such evidence originated from studies on X-linked genes in females showing that only genes from X-chromosome were expressed in a tumour

(Bodmer, 1994). However mutations can occur not only at the stem cell level but also at the somatic cell level. Each mutation that occurs at the somatic cell level is an independent evolutionary process, making each tumour distinct. Because many other mutations may occur within a developing tumour, each one has a potential selective advantage for inheritance (Bodmer, 1994). Many studies until now have given clear-cut evidence of specific mutations and translocations in genes leading to cancer. The work of Varmus and Bishop initially and others at a later stage showed that the transforming ability was determined by genes homologous to normal nuclear genes. Such work has led to the discovery of dominant oncogenes (Bodmer, 1994).

There are two categories of genes and the proteins they encode describe the functional features that drive carcinogenesis at molecular level. These are, oncogenes and suppressor genes. Oncogenes are normal, regulatory genes whose activity is increased as a consequence of genetic alterations. Oncogenes can be activated by mutations e.g. in a coding sequence (a codon) that generates an altered product. Alternatively chromosomal rearrangement can result in increased production of a normal protein or fusion protein altering its biological activity. An example of such chromosomal rearrangements specific for a disease is the formation of the tpr-met oncogene in gastric cancer (Sasajima *et al.*, 1979, Soman *et al.,* 1991; Liang *et al.,* 1996). In 1984 Cooper *et al.,* detected a new oncogene in a human osteosarcoma cell line (MNNG-HOS). This oncogene has been shown to result from a rearrangement between the 5' part of the tpr gene located on chromosome 1 and the 3' region of a gene located on chromosome 7, the c-met proto-oncogene. This new oncogene has been denominated as the tpr-met oncogene. The most characteristic feature of the tpr-met gene is its ability to activate through rearrangement, proto-oncogenes from the TK (tyrosine kinase) receptor family. The c-met proto-oncogene, codes for the hepatocyte growth factor- scatter factor (HGF-SF), transmembrane TK receptor. HGS-SF is a multifactorial cytokine, which when binding to its receptor on epithelial and endothelial cells induces TK transduction pathways, which affects cell

motility, cell proliferation, morphogenesis, tissue invasion and angiogenesis (Wicker *et al.,* 1995). The oncogenic tpr-met RNA is 5.0Kb hybrid transcript encoding a 65 KDa fusion protein. The Tpr-met hybrid protein has tyrosine kinase activity and is constantly phosphorylated. It has been shown by Kamikura *et al.*, 1995 that the most highly phosphorylated tyrosine residue is the (Y489) in the carboxy terminus of the tpr-met oncoprotein and that this residue is essential for biological activity of the tpr-met oncoprotein. Other residues are involved but not to the same degree. The potential tyrosine phosphorylated sites contained in the met receptor which is a 190kD heterodimer consisting of a 45kDa extracellular α subunit and a 140kDa β subunit that spans the membrane. The tpr domain contains a leucine zipper that mediates dimerization and activation of the Met receptor tyrosine kinase, following ligation with HGF-SF, leading to activation of oncogenes (Kamikura *et al.,* 1995). The tpr-met oncogene has been reported to be involved in tumourogenesis. Liang *et al.,* demonstrated that the expresion of tpr-met leads to the development of mammary tumours in transgenic mice and suggested that tpr-met expresion may be involved in the development of human tumours. Indeed in 1991 Soman *et al.,* observed amplification and over-expression of the c-met gene in human gastric cell line GLT-16, suggesting a possible role of met oncogene in gastric tumourogenesis. The tpr –met RNA expresion was frequently found in gastric carcinoma and in pre-neoplastic tissues.

Helicobacter pylori infection it has been established as a possible factor for development of gastric cancer. Correlation of tpr-met and *Helicobacter pylori* infection has not yet been established. In different cases of gastric cancer studied by Soman *et al.,* including superficial gastritis (SG) and carcinoma (CA), tissues showed elevated expression of tpr-met RNA but an absence of H*elicobacter pylori*. In other cases studied, the H*elicobacter pylori* infection was present but an absence of tpr-met rearrangement (Fox *et al.*, 1989; Correa *et al.*, 1989). It is believed that tpr-met gene is expressed in early stages of gastric cancer in superficial gastritis where one of the early morphological

changes observed is inflammation, followed by atrophy or gland loss and involving regenerative hyperplasia (Fox *et al.*, 1989; Correa *et al.*, 1989, Soman *et al.*, 1991). Thus, tpr-met may be expressed in early stages as a consequence of inflammation where tpr-met RNA expression continues through the later stages including chronic atrophic gastritis (CAG), intestinal metaplasia and carcinoma. However, in a Japanese study examination of tpr-met RNA expresion by RT-nested PCR in similar cell lines demonstrated lack of rearranged tpr-met mRNA (Osaki, 1996). Therefore the issue of tpr-met and gastric cancer is still controversial. Other studies have reported similar rearrangements leading to the activation of BCR-ABL in the Philadelphia chromosome translocation in CML (Haisterkamp *et al.,* 1986) and the TRK gene (Martin *et al.,* 1986). The function of many metabolic pathways may be affected as a result of oncogene activation. These include membrane receptors, signal transduction, and apoptosis and gene transcription. Some other examples of oncogenes are the ras, erb-B2, and myc genes, but there are many others.

The other types of genes involved in carcinogenesis are the suppressor genes. Suppressor genes encode for inhibitory proteins whose function is lost in cancers. Both genes copies must usually be lost in a diploid cell before an effect is to take place. This type of suppressor is recessive. Examples of known suppressor genes are the Bcl-2 and the rb genes. An exception to this rule is the p53 suppressor gene in that mutation in one allele generates an abnormal p53 protein that inactivates the normal product of the other allele. This type of mutation is defined as dominant negative. Suppressor proteins may be inactivated by binding to other proteins or by phosporylation or mutation. RNA viruses carry an oncogene coding for proteins responsible for causing cancers. Carcinogenic RNA viruses such as (HIV) influence the host genes by insertional mutagenesis. Carcinogenic DNA viruses such as (EBV) carry suppressor genes that code for suppressor binding proteins (i.e. LMP1) that bind to host proteins (Rugge & Genta, 1999). Cell proliferation and death pathways can be affected by suppressor genes, which lead to uncontrollable proliferation and formation of tumours (Steel, 1994). Oncogenes and suppressor genes co-

operate in the genesis of cancers but the exact contribution of each of the known susceptibility genes to cancer is unclear. However it seems that the high risk genes in some common cancers have been identified. Examples are hMSH2 and hMSH1 genes in colon cancer and in endometrial cancer (Easton, 1994; Stanbridge, 1990).

2. ANATOMICAL ASPECTS

ANATOMY OF THE OESOPHAGUS

The oesophagus can be found between the pharynx and the stomach. It is approximately 25 cm long and lies just behind the trachea. The oesophagus ends at the stomach passing through an opening in the diaphragm. This is known as the oesophageal hiatus. The main function of the oesophagus is to transport food from the pharynx to the stomach. The oesophageal wall is thick consisting of four layers mucosa, submucosa, muscularis and adventitia, common to the digestive tract (Fig 1). Like most parts of the digestive tract, the oesophageal muscle has an outer longitudinal layer and an inner circular layer but it differs from the other parts of the digestive tract in that it consists of skeletal muscle in the superior portion and smooth muscle in the inferior portion of the oesophagus. The regulation of movement of material into and out of the oesophagus involves coordination by the upper oesophageal sphincter and lower oesophageal sphincter. The mucosal lining of the oesophagus is made up of moist, stratified, non-keratinising squamous epithelium, and numerous mucus glands produce thick lubricating mucus.

HISTOLOGY OF THE OESOPHAGUS

Mucosa

The mucosa is the inner most layer that consists of three layers.

- The mucous epithelium, which is moist, non-keratinising, stratified squamus epithelium
- The lamina propria, a loose connective tissue
- The muscularis mucosa, a thin smooth muscle layer

Submucosa

The submucosa is a thick connective tissue layer that contains nerves, blood vessels and small glands. It lies beneath the mucosa. The submucosal plexus is formed from the nerves of the submucosa.

The muscularis

The muscularis is the third layer that consists of an inner layer of circular smooth muscle and an outer layer of longitudinal smooth muscle except in the upper oesophagus where the muscles are striated and in the stomach where there are three layers of smooth muscle. A nerve plexus known as the myenteric plexus, consisting of nerve fibres, and parasympathetic cell bodies, lies between the two layers. The submucosal and myenteric plexuses comprise the intramural plexus that is extremely important in the control of movement and secretion.

Serosa or adventitia

Serosa or adventitia is the fourth layer consisting of connective tissue. The term Serosa applies when a portion of the digestive truck protrudes into the peritoneal cavity, whereas the layer is called "adventitia" if the outer layer of the digestive tract is derived from adjacent connective tissue. Adventitia is present in the oesophagus and the retroperitonial organs. The key feature is that the oesophagus lacks a serosal covering (Seeley *et al.,* 1992; Sherwood, 1993).

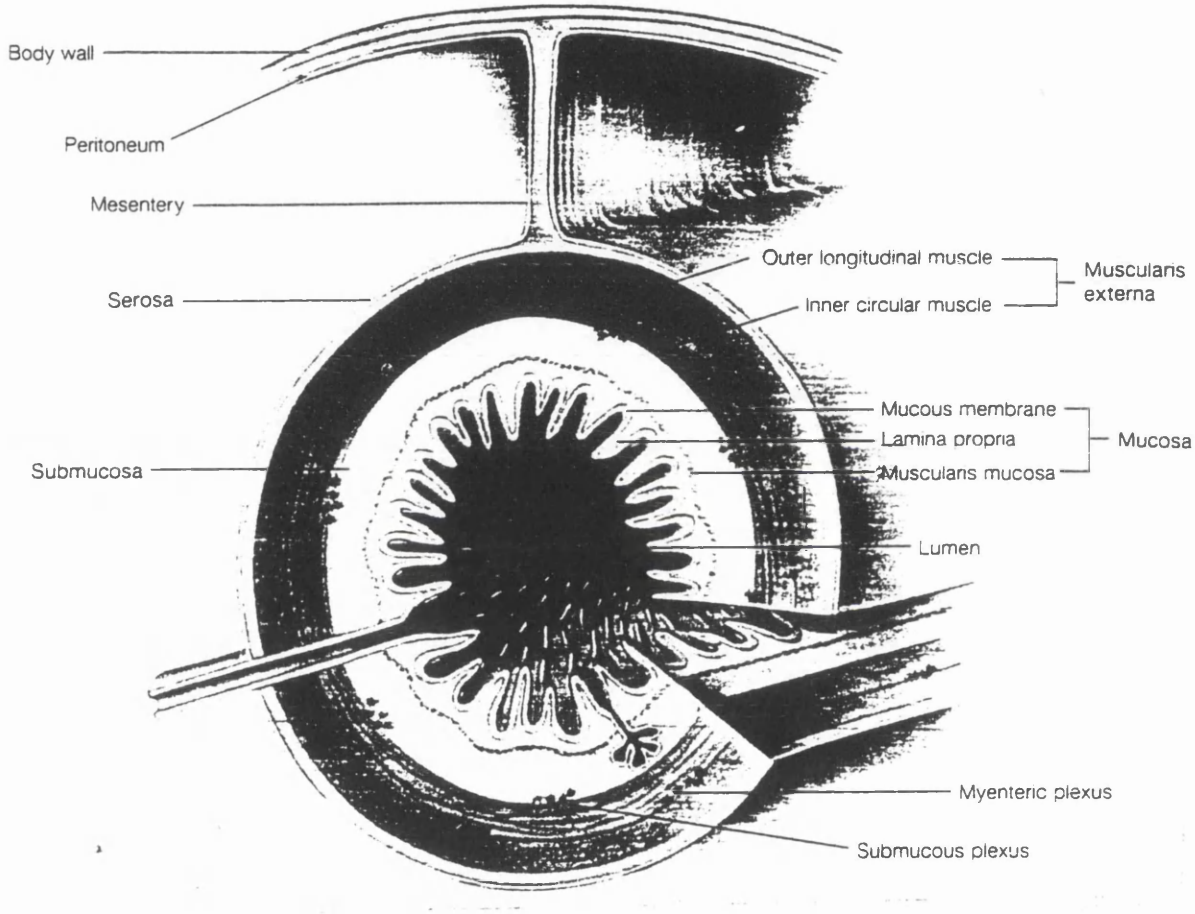


Fig. 1. The layers of the digestive tract wall.

The digestive tract wall consists of four tunics, the mucosa, submucosa, muscularis and serosa or adventitia (Sherwood, 1993).

ANATOMY OF THE STOMACH

The lower part of the oesophagus, which opens into the stomach, is known as the gastro-oesophageal junction opening (Fig 2). The region of the stomach below the gastro-oesophageal junction is known as the cardia. The portion of the stomach left and superior to the cardia is known as the "fundus" whereas the largest portion of the stomach is the "body". The body turns to the right forming a greater curvature and a lesser curvature. The lower part of the body narrows to form the antrum, which is leads to the pylorus. The "pyloric opening" is the opening between the stomach and the duodenum, and is surrounded by a thick ring of the smooth muscle known as the pyloric sphincter.

HISTOLOGY OF THE STOMACH

Unlike the oesophagus the outer layer of the stomach is the serosa. It consists of an inner layer of connective tissue and an outer layer of mesothelium. The muscularis propria of the stomach consists of three sub-layers: an outer longitudinal followed by a middle circular layer and an inner oblique layer. The mucosa and submucosa form folds known as "rugae", when the stomach is empty. Rugae are elastic allowing the mucosa and submucosa to stretch when the stomach is filled. Columnar epithelium lines the stomach where the mucosal surface forms tube-like pits. Gastric pits form the opening where the gastric glands lie. Five groups of the epithelial cells exist:

- The surface mucus cells that produce mucus, located on the surface of the lines and gastric pits. The remaining four are the glands.
- The mucus neck cells that produce mucus,
- The parietal cells that produce hydrochloric acid and the intrinsic factor,
- The chief (zymogenic) cells that produce pepsinogen,
- The endocrine cells that produce regulatory hormones (Seeley *et al.*, 1992; Sherwood, 1993).

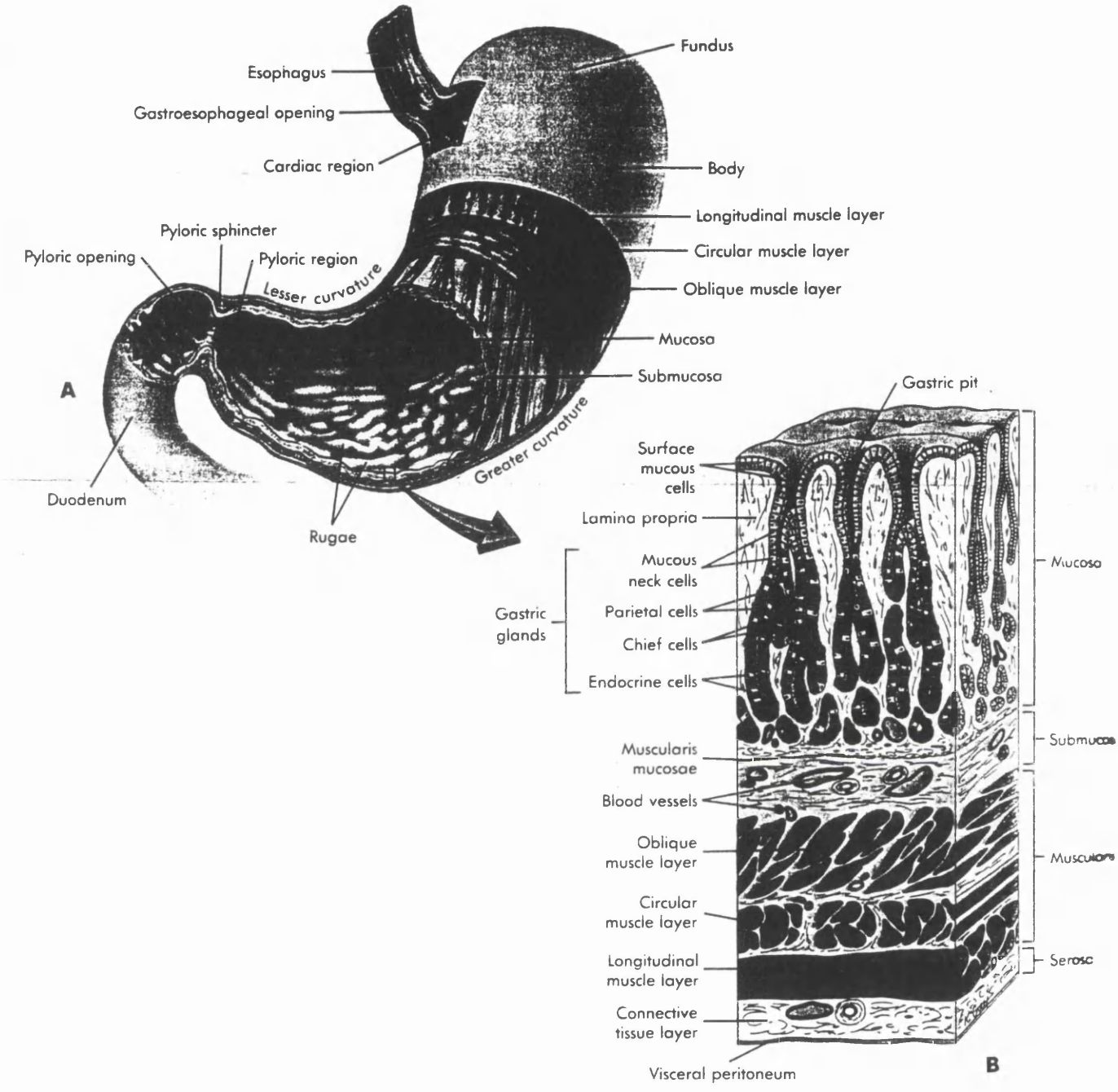


Fig. 2. Diagrammatic representation of the stomach anatomy.

A: The muscular layers and the internal anatomy are revealed.

B: This is a section of the stomach wall that illustrates its histology, including several gastric pits and glands (Seeley *et al.*, 1992).

3. BARRETT'S OESOPHAGUS

The first description of Barrett's oesophagus was from Norman Barrett in 1950 (Barrett, 1950). Ulcers in the distal part of the oesophagus lined by columnar epithelium were observed. It was believed that the ulcerated columnar lined tissue was a kind of tubular segment of the upper-stomach, a characteristic of patients with congenitally short squamous lined oesophagus. It was not until 1953 that Allison and Johnston reported the confirmation that columnar epithelium lines the distal oesophagus and suggested that Gastro-Oesophageal Reflux Disease (GORD) was involved in the pathogenesis (Navaratnam & Winslet, 1998). Since 1970 the incidence of adenocarcinoma of the oesophagus has increased in western countries at a vigorous rate (Bytzer *et al.*, 1999; Blot *et al.*, 1991). Barrett's oesophagus represents the replacement of ≥3 cm squamous epithelium of the distal tubular oesophagus by metaplastic columnar epithelium. Three types of columnar epithelium are known:

a) Specialised columnar epithelium that exhibits a villiform surface, mucus glands and intestinal type goblet cells.

b) An atrophic gastric fundic type of epithelium involving chief and parietal cells.

c) A junctional type epithelium similar to that of the gastric cardia.

In theory, the junction between the squamous epithelium and the columnar epithelium is located at the anatomic junction of the oesophagus and stomach. The exact location of the end of the oesophagus and stomach cannot be identified precisely therefore the proximal margins of the gastric mucosal folds are used as an indication for the oesophagus gastric junction. In almost 100% of the cases the columnar Barrett's mucosa consists of specialised epithelium with incomplete intestinal metaplasia. It has been observed that columnar epithelium with intestinal metaplasia will predispose to adenocarcinoma (Hameeteman *et al.*, 1989). The origin of such change is not precise.

AETIOLOGY & PATHOPHYSIOLOGY OF BARRETT'S OESOPHAGUS

The main aetiological factor is GORD. The lower oesophageal sphincter relaxes abnormally due to dysfunction, causing reflux of gastric acid (Gastro-Oesophageal Reflux). Other contributory factors in Barrett's oesophagus could be the presence of a hiatus hernia or ulcerations or the oesophageal epithelium's ability to resist refluxate. A long period of exposure to gastric acid reflux or to bile induces abnormal changes to the epithelium lining of the oesophagus. However, not all patients with GORD develop Barrett's oesophagus and exhibit complications that leading to adenocarcinoma (Navaratnam *et al.*, 1998). The sequential progression of change is from normal stratified squamous epithelium, to metaplastic columnar epithelium, then to dysplasia, which leads to carcinoma *in situ* and eventually invasive carcinomas, although this process is not solely due to GORD. Environmental risk factors such as smoking and ageing have been identified to a play role in the development of the disease (Van den Boogert *et al.*, 1998).
However, free radical generation such as super oxide anions, hydrogen peroxide and hydroxyl radicals is now known to play a role in the development of such cancers (Navaratnam *et al.,* 1998).

MOLECULAR ASPECTS OF BARRETT'S OESOPHAGUS

Multiple genetic alterations affect both cellular and proto-oncogenes and tumour suppressor genes, during the sequential development and progression of Barrett's oesophagus. Different histological forms of oesophageal carcinomas suggest that a spectrum of genetic alterations are involved in different tumour types. The abnormalities that result in the p53 gene are the most frequently occurring. p53 is located on chromosome 17. Inactivation through mutation or allelic deletion, contributes to the onset of tumours. The p53 protein is closely linked with tumourogenesis. Loss of heterozygosity (LOH) that occurs in tumour types, and following mutations in the gene, produces an aberrant protein that inhibits the activity of the normal allele. In 50% of oesophageal

cancers, the p53 gene is abnormal (Meltzer *et al.*, 1991), and point mutations are involved. The missence type mutation is the most common found in oesophageal cancer although nonsense mutations have been described (Huang *et al.*, 1993; Imazeki *et al.*, 1992). Mutations on p53 that are associated with the oesophagus can be caused by genotoxic factors such as aflatoxin B1, benzopyrene, nitrosamines and infection by human papiloma virus (HPV) of the oesophagus. In Barrett's oesophagus the frequency of p53 mutation increases with the grade of dysplasia (Blount *et al.*, 1991). Suppressor p53 mutations in Barrett's oesophagus have a clonal origin. An early p53 mutation could result in functional loss of other tumour suppressor genes such as the rb gene, the MCC gene and the APC gene, found mutated in colon cancer (Younes *et al.*, 1993). Allelic deletions involving tumour suppressor genes are important in formation and progression of oesophageal cancers. Oncogenes and growth factors are now known to be involved in Barrett's oesophagus development. Oncogenes encode for growth factors (GF), their receptors and other molecules involved in the signal transduction system mediated by a tyrosine kinase pathway. An abnormality in oncogene expression leads to over expression of GF and their receptors, contributing to abnormal cell proliferation and cancer (Gousting *et al.*, 1986). Oncogenes and GFs are also involved in angiogenesis. Angiogenesis is a process of blood vessel development, important for tumour growth. There is a correlation between EGFR and the degree of dysplasia. The TGFa that is a homologue protein of the EGF and the EGF ligands of the EGFR tumour cells have been reported over expressed in Barrett's oesophagus (Al-Kasspooles *et al.*, 1993) and in adenocarcinomas (Filipe *et al.*, 1993). Al-Kasspooles *et al.*, has also reported that EGFR is co-amplified with erbB2 proto-oncogene. The erbB2 proto-oncogene encodes for a transmembrane tyrosine kinase receptor highly homologous with EGFR, which probably explains the co-amplification. TGFa has been found to play a role in the development of adenocarcinoma of the oesophagus. An increase in TGFa and EGFR expression occurs through the progression of non-metaplastic epithelium, to dysplastic and finally the development of carcinomas (Filipe *et al.*, 1993).

Such studies indicate the wide spectrum of genes involved and the complexity of the oesophageal carcinomas. Other oncogenes such as the H-ras are also known to be involved in Barrett's dysplastic mucosa, and adenocarcinoma, but are not found in nondysplastic Barrett's metaplasia (Abdelatif *et al.,* 1991).

4. GASTRIC CANCER

Gastric cancer is a major world problem, despite advances in early diagnosis and treatment. Since 1930 there has been a significant decline in its incidence but it still remains the second most frequent cancer but not in UK (Correa & Chen, 1994). The events involved in transformation of gastric mucosa and progression are poorly understood. Epidemiological studies have identified many factors possibly related to the development of gastric cancer. Factors that may be related are *Helicobacter pylori* infection, diet, insufficient intake of vitamins A, C, E and flavinoids, environmental toxins, geographic location, tobacco, alcohol, Ebsten-Barr Virus (EBV) infection, atrophic gastritis, intestinal mataplasia, presence of post surgical gastric remnant and genetic predisposition. Gastric carcinoma is divided into two general clinicopathological patterns (Lauren, 1965).

a) Intestinal type, or well differentiated carcinoma

b) Diffuse or poorly differentiated carcinoma

Over 95% of the gastric cancers are adenocarcinomas derived from the epithelium of the stomach. The intestinal-type lesions follow a progressive sequence of steps from chronic gastritis to gastric atrophy, then to intestinal metaplasia, progressing to dysplasia, then to early carcinoma and then finally to invasive carcinoma that leads to metastasis. Metastasis of gastric cancer is often accompanied by lymph node metastasis. According to studies by Yasuda *et al.,* (1999) the tumour size and depth of submucosal invasion serve as a simple and useful indicator of lymph node metastasis in the early stages of gastric carcinoma. However, over the past decades the major decline in gastric cancer mortality has been mostly due to a reduction into the number of cases of well-differentiated type, while poorly differentiated cancers are still a major problem, probably due to lack of precursor changes seen in the epidemic intestinal-type (Morris & Bruckner, 1997). The molecular events that lead to these two forms appear to be different. The transformation of normal gastric epithelial cells to cancer cells is complex and associated with abnormalities in oncogenes, cellular growth factors and their receptors, tumour suppressor genes and

adhesion molecules and DNA repair genes that will be discussed later. As in all other cancers, differences in molecular pathogenesis depend on the histological type of gastric cancers (Wright *et al.,* 1993).

CHROMOSOMAL ABNORMALITIES

Karyotypic analysis has shown that complex chromosomal abnormalities exist in both gastric adenocarcinoma and sarcoma. Granberg first did karyotypic analysis for gastric cancer in 1973 (Morris & Bruckner, 1997). Chromosomal abnormalities have been shown that include tetraploidy, triploidy and hyperploidy. An example of a chromosomal abnormality would be translocation on ALL-1 gene at 11p23. This has been observed in gastric cancer cell lines. (Van Dekken *et al.,* 1999). This translocation is not the only one observed in gastric cancer, as the tpr-met translocation described previously also has been identified (Soman *et al.,* 1991). In addition, deletions of gene regions have been seen in gastric cancer but not always are strongly associated. It has been found that the loss of 14q31-32.1 on chromosome 14 occurred significantly more frequently in Barrett's related adenocarcinomas of the distal oesophagus than in gastric cardia cancers (P=0.02) as reported by (Van Dekken *et al.,* 1999). Diffuse-type tumours with loss of Y and X chromosome disomy in male, and uni-parental X chromosome disomy in female patients have also been seen (Saal *et al.,* 1993). The intestinal type of cancer presents aneuploid karyotypes with frequent structural abnormalities (Saal *et al.,* 1993). However, other chromosomal abnormalities such as loss of heterozygosity (LOH) have been reported on other chromosomes such as chromosome 7. (LOH) on chromosome 7q and especially for marker D7S95 is correlated with metastasis and poor prognosis was observed (Kuniyasu *et al.,* 1994). The incidence in well-differentiated gastric carcinomas was 53% but in poorly differentiated and scirrhous gastric carcinomas were 33% (Kuniyasu *et al.,* 1994). This study lead to the conclusion that this may be a site of a tumour / metastasis suppressor gene. Similar studies have revealed the involvement of the APC gene and (the closely

linked MCC gene), the DCC gene, the k-ras gene, the p53 gene, the tpr-met gene, playing essential roles in the progression of gastric neoplasia probably due to telomeric instability (Tahara *et al.,* 1994).

Recent studies have focused upon telomers, the terminal end of chromosomes. The telomers consist of specific nucleotide repeats that are responsible for the stability of chromosomes. In cancer telomerase is turned on and this prevents the shortening of the telomers, which is probably seen in an ageing cell. Immortality is therefore conferred on the cancer cell. It has been demonstrated that reduction of telomers occurs in gastric cancer (Hiyama *et al.,* 1995). Most of the tumours with telomerase activity are large and are of advanced stages including metastasis. Patients with tumours with telomerase activity have shorter survival rate than those without (Hiyama *et al.,* 1995).

For the purpose of this project the investigation of genetic markers located telomerically of chromosome 6 was necessary. The genetic markers utilised were HLA-BQB1 within the HLA class II region, the HSP-70 and the D6S273 marker within the HLA class IV region.

TUMOUR SUPPRESOR GENES, ONCOGENES AND GASTRIC CANCER

The most commonly observed genetic abnormalities in gastric cancer are mutations and deletions of tumour suppressor genes. Most frequently the p53 gene has been identified to be abnormal in many cancers. The p53 protein acts as a DNA binding factor, regulating transcription and the control of cell entry to S – phase of the cellular cycle. It is also involved in apoptosis and terminal differentiation. Such events are important for the molecular onset of gastric cancer. In histological subtypes of gastric cancer progression towards (i.e. intestinal mataplasia, gastric adenomas, low grade dysplasia, high grade dysplasia and carcinoma) p53 mutations have been identified with increased frequency in the late stages (Rugge *et al.,* 1992). Nonsense mutations (alteration of a coding sequence for an amino acid into a termination codon), frame shifts (a mutation resulting from

insertion or deletion of a group of nucleotides that is not multiple of three and therefore changes the frame in which the altered gene is translated) and (LOH) (loss of one or two distinguishable alleles at heterozygous locus) are abnormalities that are commonly seen.

Other genes such as the APC gene that is located on chromosome 5q have been reported to be mutated in gastric cancer (Horii *et al.,* 1994). The most frequent mutations of the APC gene are nonsense mutations. The 10% of APC gene mutations are somatic mutations of hyperplastic gastric polyps, whereas 25% are adenomas. The APC gene exhibits a histologic specificity. In 60% of well-differentiated gastric carcinomas, the APC gene is mutated or completely lost, making it the most frequent alteration in this type of carcinoma. The function of the product of APC is uncertain although suspected to be involved in cell adhesion and signal transduction by binding to cell surface molecules such as E-cadherin and B-catenin. E-cadherin is mutated in 50% of diffuse type gastric cancers suggesting that it confers metastatic properties (Henning *et al.,* 1995). On chromosome 5q there is a second locus, MCC. The MCC (mutated in colon carcinoma) gene is closely linked with the APC gene and it has found to be deleted or mutated in gastric cancer (Ryu *et al.,* 1994). In contrast with the APC gene the MCC gene is found to be deleted or mutated far more in poorly differentiated cancers (23.5%) and rarely if at all in differentiated cases (Hsieh *et al.,* 1995). LOH at APC / MCC gene loci has been found in both differentiated and undifferentiated types in both early and advanced stages. Thus, LOH at MCC / APC is considered an essential genetic alteration in human gastric cancer (Tamura *et al.,* 1996). A study in a British population failed to report frequent LOH of the MCC / APC genes in gastric cancers, suggesting that tumour suppressor genes in other chromosomes may play a more significant role (Sud *et al.,* 1996). Therefore it can be concluded from this evidence that the mutational pattern of APC / MCC and p53 presents a discordance, suggesting that such cancers have a multi-centric genetic origin. Allelic loss has also been reported on 18q chromosomal site. The DCC (deleted in colon cancer) locus on chromosome 18q codes for protein homologues to neuronal adhesion molecules of the

Ig gene super family and is believed to contribute to cell adhesion and signalling. In well-differentiated gastric cancers, the DCC gene is deleted (Barleta *et al.,* 1993). Therefore the overall pattern of allele loss in gastric cancer has many similarities with the colon cancer with losses at 5q, 18q, and 17p.

Apart from tumour suppressor genes, several oncogenes may also be implicated in gastric cancer. Mutations and overexpression of the *ras* family have been observed. A significant correlation between *ras* oncogene types and geographic variations has been seen. There is a low frequency of *K-ras* mutations in western European countries, Japan and more prevalent in Italy and China where more point mutations take place (Hongyo *et al.,* 1995). *K-ras* expression is an early event in the progression of diffuse type and well-differentiated gastric cancers. *K-ras* is correlated with depth of invasion, metastasis and shortened survival. *K-ras* is probably not an indicator of neoplasia. Overexpression of *K-ras* has been observed in gastritis, benign ulcers, atypia, and dysplasia and in normal gastric epithelium near to tumour lesion. Other oncogenes, such as the *K-sam II* proto-oncogene is frequently amplified in poorly differentiated tumours and scirrhous gastric carcinomas (Yokozaki *et al.,* 1994). *K-sam II* is a member of the fibroblast growth factor receptor family (FGF). Basic fibroblast growth factor–2 (bFGF-2) is overexpressed in scirrhous carcinomas (Yokozaki *et al.,* 1994) where both FGF and bFGF correlates with large tumours, poorly differentiated, deep invasion and increased metastasis to lymph nodes. In scirrhous type stomach cancer frequent amplification of *c-met* gene has also been seen (Kuniyasu *et al.,* 1992). There is a correlation between *c-met* amplification and clinical stage. In well-differentiated adenocarcinomas *c-met* behave as a suppressor gene (Kuniyasu *et al.,* 1992). A rearrangement of *c-met* and tpr oncogene to produce a 65 KDa tpr-met fusion onco-protein has been reported in gastric cancer (Soman *et al.,* 1991) as described previously.

Expression of c-erbB2 gene has also been reported in gastric cancers. c-erbB2 linked with large primary serosal tumours and lymphatic invasion and distant metastasis.

c-erbB2 is overexpressed in metaplastic lesions with microsatellite instability more often (64.3%) than in primary tumours without (28.6% , $p<0.05$) (Lin *et al.*, 1995). Blockage of apoptosis leads to immortalisation of cells, a phenomenon seen in cancer. The bcl-2 gene is associated with blockage of apoptosis where increased expression has been seen in colonic polyps and carcinomas and more recently in gastric epithelial dysplasia but not in normal gastric mucosa. Bcl-2 expression has not been found in Barrett's oesophagus even when severe dysplasia occurs. Overexpression of bcl-2 has been seen in poorly differentiated and scirrhous gastric carcinomas and believed to act as a dominant oncogene. In addition bcl-2 expression has been noted in gastric epithelial dysplasia as well as in chronic atrophic gastritis with intestinal metaplasia (Lauwers *et al.*, 1994). This leads to the conclusion that extensive cell survival due to inhibition of apoptosis is essential in addition to increased cellular proliferation in altered cells towards progression of gastric carcinogenesis. Other factors such as platelet-derived growth factor (PDGF), CD44 adhesion molecules, basic fibroblast growth factor (b-FGF), and insulin like growth factor (IGF- II), musin proteins (MUC1), hepatocyte growth factor (HGF) and IL-1a are all found expressed in various types of gastric cancer all acting as regulators of cell growth (Yokozaki *et al.*, 1994).

INFECTIONS RESULTING IN GASTRIC CANCER

Epstein Barr Virus (EBV) infection has shown to be a cause for the onset of small number of lymphomas. Studies have shown that of the pathologic material from gastric cancer patients EBV was found to be present in 12 % of the cases. It was found to be in large numbers of the stomach lesions that are composed of malignant epithelium and in dysplastic gastric epithelium (Gulley *et al.*, 1996). It has been suggested that EBV infects gastric epithelial cells through a reaction with T-lymphocytes carrying EBV. Immunohistochemical analysis of such lesions has shown accumulation of p53 protein but it found to be similar to EBV negative specimen. This lead to the conclusion that EBV infection does not correlate with p53 mutations during carcinogenesis. Similarly bcl-2

expresion was tested but no correlation was detected with EBV accumulation. Therefore, it is not clear yet if EBV is involved in the onset of gastric cancer (Gulley *et al.,* 1996). *Helicobacter pylori* bacterial infection is recognised as a risk factor for acute inflammatory gastritis, peptic ulceration, and lead to gastric cancer in western countries. Infection with *Helicobacter pylori* in childhood increases the risk of developing gastric cancer. *Helicobacter pylori* is not found in normal stomach but frequently found in chronic gastritis (Farthing, 1998). The correlation between *Helicobacter pylori* and gastric cancer is complex.

5. *Helicobacter pylori*

Helicobacter pylori is a gram-negative bacterium, with features such as sheathed flagella, external glycocalyx, and a G and C base content of chromosomal DNA, that enables it to be considered in the genus *Helicobacter*. Seven species are known to be associated with gastric mucosa in a variety of mammalian species and ten with intestinal mucosa. *Helicobacter pylori* has a circular genome with more than 1.6 million bp and 1,590 coding sequences. *Helicobacter pylori* has the ability to mimic the human molecular immunological structure that probably explains the ability to avoid immunological defence mechanisms and therefore the involvement in gastritis (Farthing, 1998).

EPIDEMIOLOGY OF *Helicobacter pylori*

Humans are the main host of *Helicobacter pylori* infections. The average incidence of infection is 40%-50% in the Western countries while in developing countries this increases to 90%. It is believed that in Western countries the incidence is now decreasing, significantly. The ratio for men and women developing gastric cancer, due to *Helicobacter pylori* infection has been shown to be 2:1 (Forman, 1998). The route of transmission is not yet clear. It was believed that one possible route is through an infected water supply.

Another possible route could be endoscopy. Oral-oral, gastro-oral and faecal-oral routes are other possible routes. Some studies have shown frequent isolation of strains from oral cavities but other studies found this less frequent as indicated by Rowland and Drum, 1998. Thus, water or dental sanitation cannot be considered as the main source of *Helicobacter pylori* infection but may contribute. The main route of transmission nowadays is by direct person-to-person contact (Feldman *et al.*, 1998). The age of acquisition of *Helicobacter pylori* shows geographical variation. The most frequently acquisitions occur in persons 15 years of age or younger. In adults is only 1% to 3% per annum. The overall prevalence is 30%-40% in developed countries but can be 30% to 40% in children from low socio-economic groups. In developing countries the range is from 80% to 100%. Infection in early age is essential for development of gastric cancer (Rowland & Drum, 1998).

VIRULENCE ASSOSIATED GENES

Among people with *Helicobacter pylori* infection not all develop disease. The main disorders that arise are duodenal and gastric ulceration. *Helicobacter pylori* is considered a major risk factor in the development of adenocarcinoma and mucosal associated lymphoma (MALT). Of those infected, the virulence of the *Helicobacter pylori* strain appears to be a major factor in determining, who will develop disease (Atherton, 1998). Whether all strains can cause peptic ulceration or gastric carcinoma is unclear. Certain strains with specific characteristics have been linked with disease. These characteristics are the "Vacuolating cytotoxin" (Vac) production and specific types of VacA gene alleles, the "Cytotoxin associated gene" product A (CagA) and genes within the (Cag) "Pathogenicity Island" and the ability to strongly and rapidly stimulate neutrophil degranulation. Host susceptibility may be relevant to onset of disease. There is growing evidence that the host's HLA type, and its expression are important as genetic factors of susceptibility to disease. The HLA class II gene BQβ1 *0301 has been reported to be associated with gastroesophageal adenocarcinoma in positive *Helicobacter pylori* patients (Lee *et al.*,

1996). The fact that *Helicobacter pylori* produces a protein that induces vacuolation in a variety of cultured epithelial cells first was first shown by (Leunk *et al.,* 1988). Strains producing Vacuolating cytotoxic activity were more commonly isolated from patients with peptic ulcers than without. The gene VacA (encoding the toxin) varies especially in its signal activity between different *Helicobacter pylori* strains. Cytotoxic activity and signal sequence type influenced by VacA genes type, is closely associated with peptic ulceration. Strains possessing the CagA are more commonly found in people with peptic ulceration and gastric adenocarcinoma than without. CagA is probably a marker for Cag "Pathogenicity Island" (Atherton, 1998). This includes genes responsible for increased inflammation induced by pathogenic strains. The ability of Cag A to induce inflammation can be explained by the fact that sequencing around CagA has shown, that to be of a cluster of 40 genes usually present or absent as a group. Any disruption in one gene reduces the ability of Cag A^+ strains to induce IL-8 production that acts as chemotactic factor for T-cells, neutrophils, and basophiles. Activated neutrophils release lysosomal enzymes and induce adhesion of neutrophils to endothelial cells. Also cytokines such as GRO-a, RANTES, and EMPI-a are released (Kassai *et al.*, 1999; Farthing, 1998). Cag A^+ strains usually have the VacA s1 or B genotype and they are often toxicogenic, whereas $CagA^-$ unusually has VacA s2 genotype and are not toxicogenic. The detection of Cag A^+ strains in patient's serum could be a good practical test for detection of onset of inflammatory diseases. However the interrelationship of such factors is poorly understood making it difficult to estimate their relevance (Atherton, 1998). Despite the fact that Helicobacter *pylori* strains appear to be a well known factor in the development of gastric cancer, in our study testing for positivity and / or negativity of the results obtained against Helicobacter *pylori* specific strains was not possible. This was due to the fact that such strain specific data was difficult to be obtained for the test populations tested, the experimental time within the laboratory was limited as was the range of subjects with Gastroesophagial cancers at GRI, whose DNA samples were obtained for testing. Therefore, if split in to

groups of positive or negative for specific strains this is not a fair representation of entire population for statistical purposes. However, it was necessary to mention at this point the involvement of Helicobacter *pylori* because it plays an important role in gastric carcinogenesis.

6. INFLAMMATION AND GASTRIC CANCER

The potential signs of inflammation have arisen since the first century of Common Era, when Cornelius Celsus (30_{Bc}-38_{AD}) was describing the typical reaction of tissue to microbes. Redness, swelling, heat and pain -*"rubor et tumor cum calore et dolor"* are the four common signs of inflammation. Human hosts and animals have an invisible extensive defence mechanism ready to oppose to anything-foreign otherwise known as "antigen". If the hosts defence mechanism fails to eliminate the antigen, infection occurs. If the hosts manage to eliminate the antigen, then immunity occurs. Until the 19th century there was no knowledge about inflammation. By the first decade of the 19th century, scientists were using microscopes to identify bacteria as the cause of infection and found that when introduced into the host, infection resulted (Weismann, 1992).

Inflammation, which may be acute or chronic, can be defined as the body's response to injury. Microscopically, it involves events such as dilation of arterioles, of capillaries and veins increase permeability to blood flow, exudation of fluids and plasma proteins and leukocyte migration into the inflamed area. Accumulation of leucocytes in inflamed tissue is a common event while deficiency of inflammation leads to compromised host. Recognition of host tissue as "foreign" leads to inflammatory autoimmune diseases. The characteristics of inflammation are amplified or propagated in acute and chronic inflammation, depending on recruitment of various cellular components of the immune system. Recognition occurs by specific or non-specific means. Specific recognition is mediated by immunoglobulin i.e. antibodies and/or T-lymphocyte receptors that bind to specific epitopes. Non-specific recognition involves denaturation of proteins or endotoxins that can be mediated directly by phagocytes. Binding of an antigen to a recognition component of the immune system leads to an amplification system activation and release of pro-inflammatory substances that creates the four signs that characterise inflammation. Leukocyte adherence to vascular walls increases vascular permeability, and migration to infected tissues, where leucocytes are stimulated to destroy the antigen. Finally,

macrophages, Kupffer cells, and type-A synovial lining cells can mediate the destruction of antigens. Destruction outside the phagocyte and related cell system takes place in tissue spaces and is mediated by neutrophils migrating from peripheral blood. In most cases the antigen is eliminated without clinically detectable inflammation. When clinical inflammation is observed this would suggest that the antigen was of a high dose or in an unusual location or difficult to digest. (Gallin *et al.,* 1992).

Inflammation is implicated to be involved in the pathogenesis of gastric cancer (Ernst, 1999). Evidence from studies in humans and animal models suggest that chronic inflammation of gastric mucosa is associated with *Helicobacter pylori* infection and especially with $CagA^+$ strains (Perez-Perez *et al.,* 1999). *Helicobacter pylori* produce an enzyme known as urease, which contribute to changes in mucosal permeability and facilitate the generation of chronic immune responses. Production of ammonia not only protects the organism from gastric acid but also has toxic effects on the gastric epithelium, inducing proinflammatory cytokines (Tanahasi *et al.,* 2000). *Helicobacter pylori* associated chronic gastritis, induces gastric mucosal inflammation that is characterised by surface epithelial degeneration and infiltration of the mucosa by chronic inflammatory cells such as lymphocytes, plasma cells, neutrophils and occasionally eosinophils (Dixon *et al.,* 1996). Inflammation has been implicated in development of intestinal mataplasia and mutations in oncogenes that proceed to the development of gastric cancer. Two mechanisms of induction of gastric inflammation are known. First the organism may interact with surface epithelial cells, producing direct cell damage or the liberation of proinflammatory mediators (chemokines) and secondly *Helicobacter pylori* derived products may enter the underlining mucosa, thereby directly stimulating immune response involving cytokines. Chemokines such as GROα, IL-8, RANTES, MIP-1α and cytokines such as TNFa, IL-12, IL-13, IL-16 GM-CSF, IFNγ, IL-1α/β, IL-18, IL-6, IL-7, PGE_2 and IL10, are induced during infection and activate T-cells, B-cells, neutrophils and macrophages. Chemokines have marked target cell specificity. IL-8 and GROα have specific chemotactic activity for

neutrophils where RANTES and MIP-1α have effects on monocytes and lymphocytes (Baggiolini *et al.,* 1994). Bacterial induction of epithelial chemokines involves protein tyrosine kinase pathways e.g. Mitogen-activated protein kinase (MAPK) and NF-kB activation (Aihara *et al.,* 1997). A number of transcription factor families involved in such pathways have been described recently in literature to be involved in inflammation. Activator protein 1 (AP-1), activating transcription factor 2 (ATF2), nuclear factor kB (NF-kB), nuclear factor of activated T-cell (NF-AT), signal transducer and activator of transcription STAT, and p53 are critical regulators of gene expresion in setting of inflammation (Firestain *et al.,* 1999).

Cytokine production is a balance between inflammatory (IL-1, IL-2, IL-6, IL-8, IL-18, TNFa, IFNγ) and anti-inflammatory (IL-1RA, IL-4, IL-10, IL-13, IL-16, TNFβ and PGE_2) products. Proinflammatory cytokines are induced in many infections, where anti-inflammatory cytokines are induced to maintain the balance. In inflammatory response due to *Helicobacter pylori* infection all the above pro-inflammatory cytokines are induced (Firestain *et al.,* 1999). However the balance tips towards the onset of inflammation. That is probably due to insufficient anti-inflammatory cytokine response or could be due to a number of genetically aberrant cytokines (e.g. TNFa, IL-1, IL-6) and aberrant transcription factors that are involved in protein tyrosine kinase signal transduction pathways that regulate gene expresion in inflammatory conditions (Firestain *et al.,* 1999). The bacteria reside in the lumen of stomach where most of the immune material resides, at the same site and thus a specialised immune response is expected to take place. This does not happen. Thus, the genetic level of such interactions must have a specific role in the induction of gastric inflammation. Inflammation due to *Helicobacter pylori* is still a mystery. Most individuals play host to *Helicobacter pylori* throughout their lives, but have never obtained protective immunity. It has been found that epithelial cells are capable of transducing the signal of infection to host so receptors of the epithelium can stimulate production of IL-8 and in turn activation of neutrophils, macrophages, stem cells and cells involved in

adaptive immune response such as B cells, T-cells and plasma cells. Molecular analysis of gastric mRNA has produced evidence that infection with *cag*+ strains is associated with increased transcription of IL-8 (Yamaoka *et al.,* 1996). This evidence shows that IL-8, a potent neutrophil chemokine, may be particularly important in gastric inflammation. Also the *cag*+ bacterial infection may reflect strain specific mucosal cytokine and chemokine responses. Mononuclear phagocytes serve as an important source of immune response to pro-inflammatory mediators and as antigen presenting cells involved initiation of specific immunity. It has been shown in vitro, that *Helicobacter pylori* soluble proteins will activate peripheral blood monocytes leading to increased expresion of HLA-DR and IL-2 receptors and the production of IL-1and TNFa (Mai *et a l.,* 1991). In addition endoscopic antral biopsies *in vitro* have shown increased secretion of IL-1β, TNFa and IL-6 in infected subjects with *Helicobacter pylori.* All three cytokines are predominantly derived form macrophages and have inflammatory actions and are involved in leukocyte activation (Noach *et al.,* 1994; Crabtree *et al.,* 1991). The inflammatory cells when activated produce inflammatory mediators to induce oxidative stress in the vicinity of the gastric epithelium. Oxidative stress can be neutralised by antioxidants such as vitamin C. However the level of antioxidants during infection is low in gastric juice. Elevated levels of antioxidants due to reactive oxygen (RO) produced by *Helicobacter pylori* in gastric epithelium can cause abnormalities to many genes and induce DNA damage (Ernst *et al.,* 1999).

Epithelial cell turnover is affected by inflammatory response to *Helicobacter pylori.* Increased epithelial cell proliferation as well as cell death by apoptosis has been described in literature. Apoptosis is a regulated process of cell death that is initiated by inflammatory mediators such as TNF, INFγ and *Helicobacter pylori.* Immunohistochemical studies have shown elevated accumulation of $CD4^+$ T- cells in gastric mucosa during infection. T- helper cells are classified into two types depending on the types of cytokine they produce. Th1 and Th2. Th1 lymphocytes secrete IL-2 and IFNγ and mediate cellular immune responses where Th2 produce IL-4, IL-5 and IL-10 which help in B-cell activation

and antibody production e.g. IgA (Seder, 1994). The IFNγ -Th1 cells are mainly seen in gastric mucosa (Karttunen *et al.,* 1995) and increased mucosal mRNA expression of IFNγ and IL-12 suggesting that Th1 response is predominant (D'Elios *et al.,* 1997). Th1-cells can cause problems because they are pro-inflammatory. IFNγ can cause an increase of expression of MHC and accessory molecules, important in anchoring and activating immune inflammatory cells. IL-8 production may be increased from gastric epithelium resulting in elevated recruitment of neutrophils. Studies have identified materials that adhere to *Helicobacter pylori* and are identical in size to HLA class II molecules. The expression HLA class II molecules as potential receptors for *Helicobacter pylori* correlates with the binding of *Helicobacter pylori* to gastric epithelium and it is suggested that the *Helicobacter pylori* – HLA class II complex is responsible for apoptosis induction by *Helicobacter pylori.* Increase of IFNγ release can cause increase of HLA class II expresion. IFNγ -Th1 cells are known to be elevated in gastritis. If HLA class II are potential receptors for *Helicobacter pylori* this could facilitate colonisation, modulate signalling from bacterium to host via IL-8 and contribute to expression of epithelial damage. If this occurs then fibrosis and metaplastic tissue will be formed, leading to gastric cancer. However there is no clear evidence to suggest that these events are specifically involved in the development of gastric cancer (Fan *et al.,* 1998). This kind of cycle of immunological events can contribute to mucosal damage. It is believed that there are many precursors that can cause abnormal turnover on the lining of the digestive tract including the oesophagus.

The complex cytokine network involved in inflammatory response could include regulatory cytokines such as IL-10, which may serve as a suppressor of inflammation, keeping the balance between various proinflammatory cytokines. Various cells produce IL-10, notably mononuclear phagocytes. IL-10 acts as an inhibitor of phagocytes and lymphocyte responses. Increased levels of IL-10 (secreted protein and mRNA) have been reported in *Helicobacter pylori* infected gastric mucosa with levels of IL-10 increasing with the severity of gastritis (Bodger *et al.,* 1997).

7. GENERAL ASPECTS OF THE MHC MOLECULES

Major Histocompatibility Complex (MHC) is a general name describing genes encoding antigens that were first discovered in graft compatibility studies. Such antigens are known as Histocompatibility antigens. In many species the MHC has different names. In humans it is named as Human Leukocyte Antigens (HLA), where as in mice it is named H-2.

The HLA system is composed of genes that control a variety of immune functions and influence the susceptibility of more than 40 diseases, including cancer. In normal human cells over 50 genes have been identified that determine recognition of foreign proteins by T-cells. These genes have been identified mainly through their role in coding for proteins involved in tissue transplantation, sometimes referred as transplantation antigens (Rigas, 1996).

The MHC antigens are cell membrane glycoproteins that are classified into class-I and class-II. The major loci of HLA within the class-I region are HLA-A, HLA-B, and HLA-C and within class-II are DP, DQ and DR. Between DQ and DP are loci coding for transporter protein such as (TAP) that participate in selection of peptides available for binding (Fig 3).

There are other genes among class-I loci that are named non-classical class-I loci. These are, HLA-E, HLA-F, and HLA-G. Pseudogenes (HLA-H and HLA-J) that do not lead to protein are incorporated within Class-I. Pseudogenes have been identified in class-II (Rigas, 1996). There are some other genes between the class-II locus and class-I locus composing the class-III locus. Class-III locus and class-II locus will be discussed in detail later due to its complexity and importance to this work.

MHC occupies a segment of approximately 4 Mbp on the short arm of chromosome 6 and contains the highly polymorphic class-II genes, and class-I genes. These genes encode for polymorphic cell surface proteins that are involved in presentation of antigens (Ags) in immune responses. In man these two clusters are separated by 1100Kb of DNA,

termed HLA class-III. Among them the TNF, lymphotoxin α and lymphotoxin β, the HSP genes, the C2, C4A, C4B, complement factors, and the two steroid 21-hydroxylase (21-OhaseA and 21-OhaseB) genes with immune related functions are forming the HLA class-III locus as shown in (Fig 3) (Roit *et al.*, 1996; Carroll *et al.*, 1987).

The HLA genes are located in a 3600Kb region of chromosome 6. Both HLA class-I and HLA class-II molecules are heterodimeres. HLA class-I consists of a 45-Kda transmembrane α chain, which is associated non-covalently with β2-microglobulin. The gene encoding β2-microglobulin is located on chromosome 15. HLA Class-I molecules function in antigen presentation to CD8+ lymphocytes and are involved primarily in cell-mediated cytotoxicity. HLA Class-II molecules are composed of a pair of non-covalently linked α and β chains. Chain α is ~ 32 Kda whereas chain β is 28 Kda. Both chains of the class-II heterodimer are inserted into the cell membrane whereas in class-I only chain α is inserted (Fig 4). HLA molecules are members of the immunoglobulin Ig gene family. Both chains of the HLA class-II contain two external domains ~90-95 amino acid long. The membrane domains α2 and β2 are homologous to Ig constant region domains and present a disulfide loop similar to that in Ig. The N-terminal (α1 and β1domains) is the major site of variation between the different HLA class-II alleles were the α2 and β2 domains show little variability between alleles (Fig 4). Another difference between HLA class II and HLA class-I is that in HLA class-I the membrane distal domains are encoded by one chain α were in HLA class II separate α and β chains are present (Roit *et al.,* 1996; Gregersen, 1989).

Some of these genes as in (Fig. 3) with immune related function are believed to be relevant to diseases such as gastric cancer (Lee *et al.,* 1996) and maybe Barrett's oesophagus. This research project focused on the DQB1 locus in the HLA Class II region, the HSP-70 locus and the D6S273 marker within the HLA Class III region to investigate the development of gastric cancer and Barrett's oesophagus.

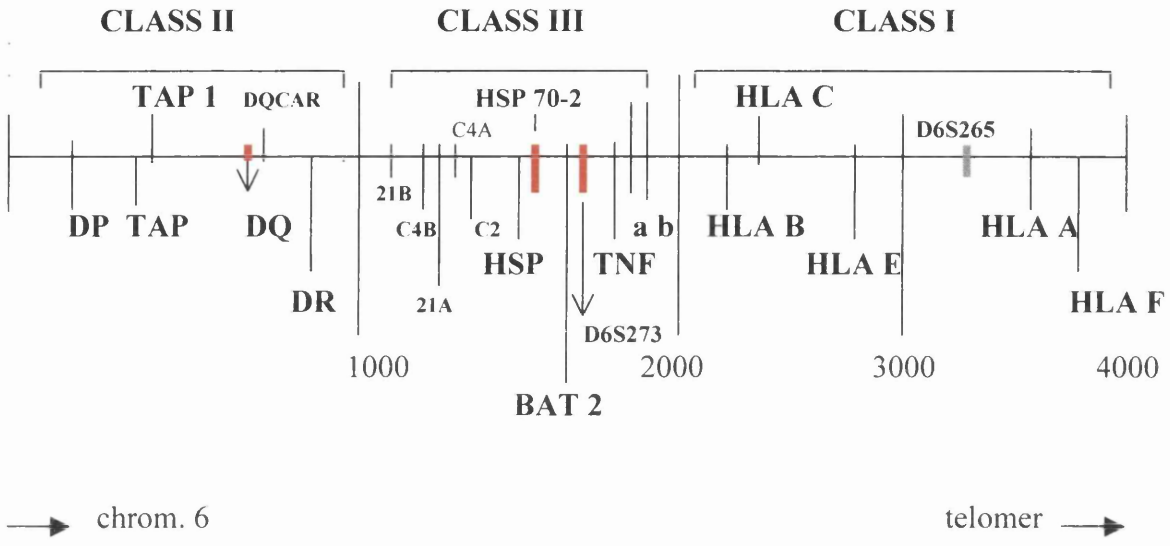


Fig 3. Genetic organizations of human MHC class I, class II, and class III and related loci telomeric on chromosome 6. The map presents a subset of loci of interest (red colour) in the region. The map is not to scale. The approximate distances are given in kilobases.

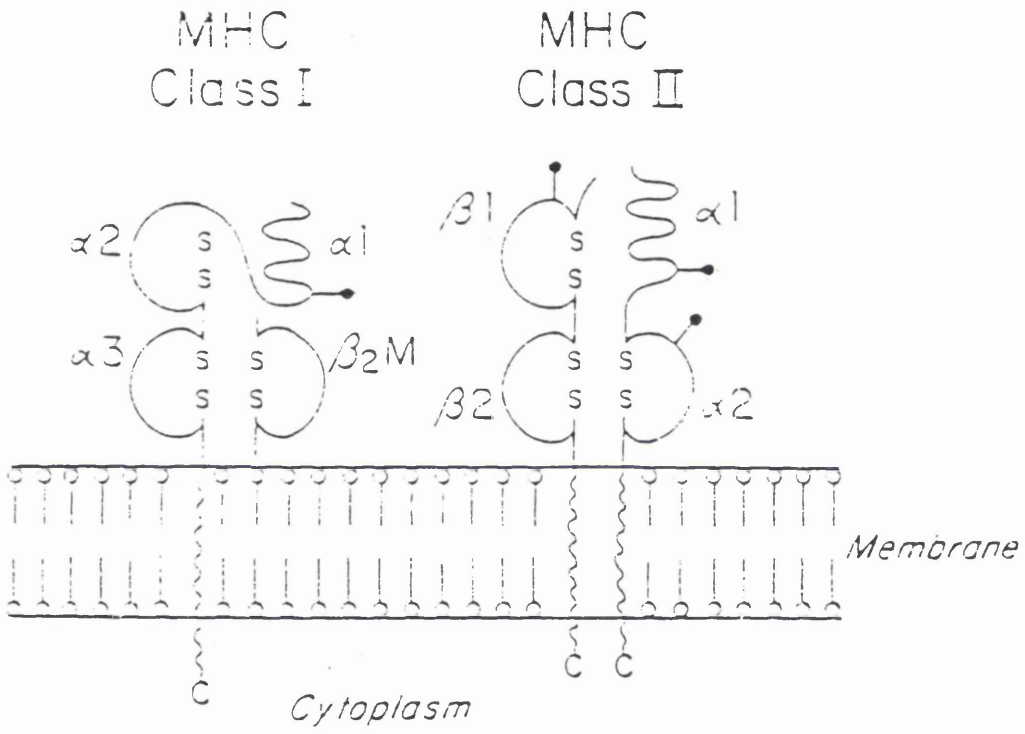


Fig 4. Schematic descriptions of the human HLA class I and class II binding domains as adopted from (Gregersen, 1989).

The T cell receptor is generally composed of an "α" and a "β" polypeptide chain. Each chain is about 280 amino acids long and has a large extracellular part that is folded into two Ig-like domains, forming the binding domain that has significant structural differences between the two classes.

THE MHC CLASS II GENES

The HLA-D region encodes for at least six α and ten β chain genes for MHC class II as demonstrated in (Fig 5). The major expressed products of the human class II are encoded by the three loci: DR, DQ and DP. The DR family comprises a single α gene (DRα) and up to nine β genes (β1-9) including pseudogenes e.g. ψβ (Fig 5). The DQ and DP families, each have one expressed gene for α and β, and an additional pair of genes, which may not be functional. The DQ subregion contains a set of genes designated DXα and DXβ that are not pseudogenes but have not been found to be expressed (Jonssosn *et al.,* 1987). Non-functional pseudogenes are also included in the DP subregion designated as ψα and ψβ (Fig 5). Additionally between the DQ and the DP subregion the DZα and DOβ genes are located. The DR, DQ, DP, α chains associated in the cell primarily with β chains of their own loci. For example the DQA1 and DQB1 encode the HLA-DQ antigens. Several gene arrangements occur within the DR locus and it is believed to be more polymorphic than the DQ and DP loci (Gregersen, 1989). In the class II region, the various heterodimeres molecules are encoded in the same genetic region that contrast to other molecules such as T cell receptor, haemoglobin or Class-I molecules that each chain is encoded in different chromosomes. The functional significance of the localization of α and β chains to the same genetic region rather than to a different genetic region is not known. However, the localization of class II α and β chains correlated with the structure and function of the molecules formed. Sequencing of the MHC has revealed that the β chain genes of DR subregion are more closely related to each other than the β chain genes of the DQ subregion. The DRβ1 and DRβ3 coding regions are over 90% similar (Curtsinger *et al.,* 1987) whereas DRβ1 and DQβ chains have 70% sequence similarity (Custafsson *et al.,* 1987). This leads to the conclusion that there is a restricted ability of β chains to form α / β heterodimeres with α chains encoded in the same subregion.

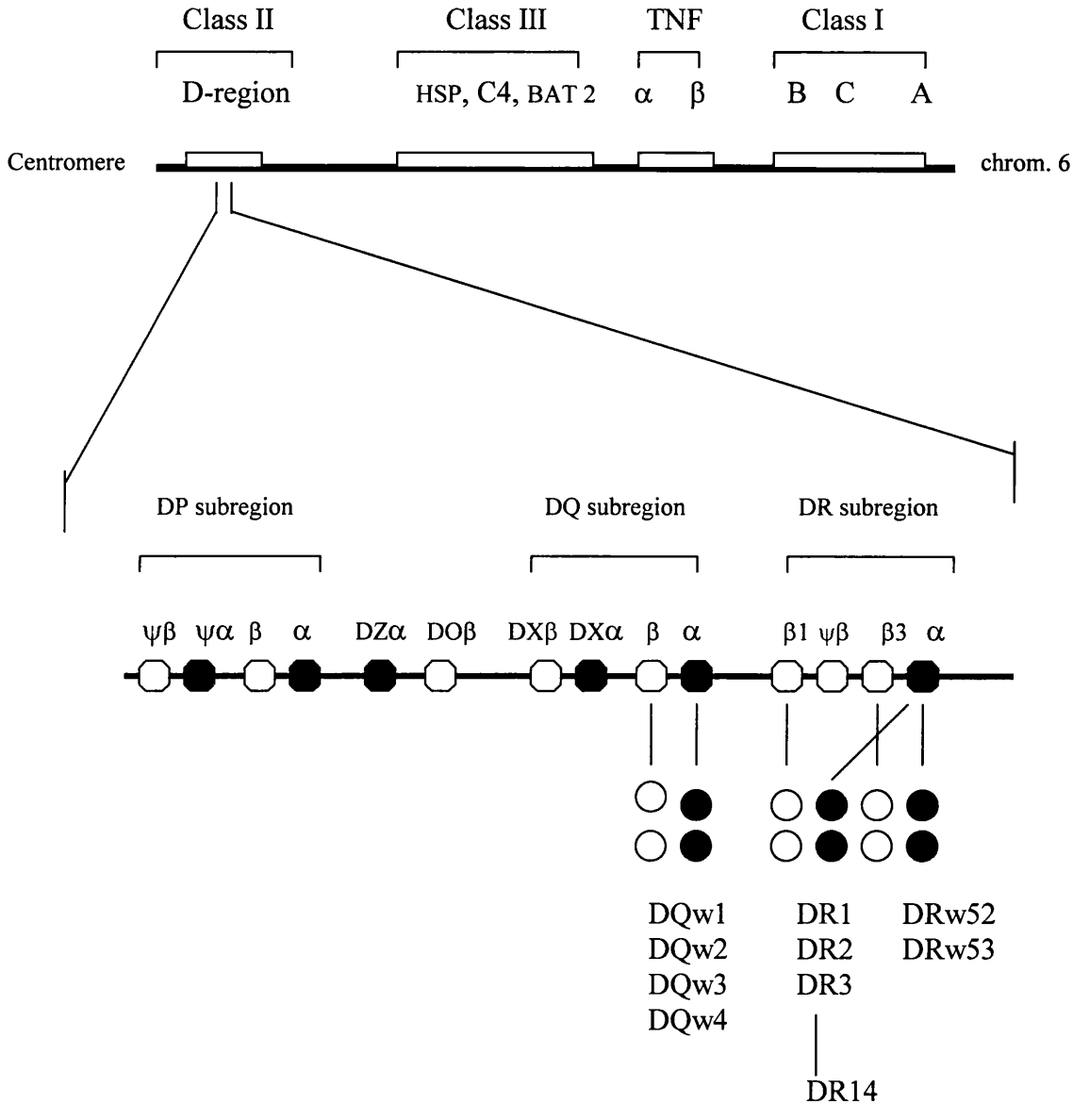


Fig 5. Schematic description of the HLA D region on Chromosome 6.

The three major polymorphic allelic series are: DR1-14, DRw52, DRw53 and DQw1-DQw4. The less polymorphic sub-regions of DP locus are also shown. Pseudogenes are designated with the letter ψ (Gregersen, 1989).

The DRα chain is identical with minor exceptions to all individuals and thus does not contribute to allelic variability of the DR molecules (Trowsdale *et al.,* 1985). Although the DQ α and β chain genes that encode the DQ series, are variable in sequence and it is believed that theβ chain is responsible for polymorphism within DQw2, DQw3 and DQw4 (Trowsdale *et al.,* 1985).

MHC polymorphism is concentrated in the peptide-binding cleft. The major function of MHC molecules is the presentation of antigens that is characterised by an extreme degree of polymorphism of the molecules encoded within it. Polymorphism is not thought to be spread evenly throughout the MHC but is focused more within class-II molecules rather than class-I, where the extent of variability depends on the sub-region and on the polypeptide chain. For example, most polymorphisms occur in DRβ rather than in DQβ chains, while DPβ chains are even less polymorphic. DQ α is polymorphic whereas DRα chains are invariant. Thus the structural diversity in the expressed molecule increases significantly. Most of the polymorphic amino acids in class-I and class-II antigens are clustered on top of the molecule in the large groove (Fig 4) that acts as the peptide-binding site (Roit *et al.*, 1996).

The implication is that this polymorphism determines which antigen-derived peptides will be presented to the cell via their T cell receptors. Those T cells that recognise the HLA / peptide complex will become activated. This polymorphism, combined with the main role of HLA class II in regulating the immune response, is the heart of the association between HLA and diseases.

HLA alleles have been identified by serological typing and designated by a letter denoting the locus followed by a number e.g. HLA-B27. The polymerase chain reaction was applied later to reveal a greater polymorphism based on allele DNA sequence. Since then a more specific terminology is used e.g. DQB1*0301.

Another feature of the HLA is the linkage disequilibrium (non-random association) between certain pairs of alleles at different loci. If a given combination of two alleles occurs much more frequently than each one alone, then they are in linkage disequilebrium. The physical meaning is that two genes pass from generation to generation together, thus preventing the equilibrium in their frequencies that would occur if they had travelled separately through successive generations. The linkage disequilebrium can be created naturally by a strong selection effect or migration and mixture of two populations. HLA association with a disease (e.g. gastric cancer) may imply that the frequency of the allele(s) is different in the patient population than in the ethnically matched control population. The implication of such large polymorphisms on T-cell antigen recognition has a great impact on scientific trials, to identify which genes are involved in diseases and especially in the case of gastric cancer (Rigas, 1996).

DQB1 LOCUS AND DISEASE

The HLA DQB1 locus maps 110 kb centromeric to HLA DR locus and is comprised of a polymorphic HLA DQB1 gene encoding at least 22 alleles and an HLA DQBA1 gene encoding at least 12 alleles (Bodmer, 1995). The HLA DQB1 and HLA DQA1 gene products define the serological antigen families designated DQw1, DQw2, DQw3 and DQw4 (Petersdorf *et al.,* 1996). Linkage disequilebrium of the HLA DQB1, DQA1 and DRB1 alleles in defined populations can produce extended haplotypes (Petersdorf *et al.,* 1996). For example, the patterns of linkage disequilebrium found commonly in Caucasian population can be seen in (Fig 6). As shown serologically defined alleles encoded within the DQ subregion are in linkage disequilebrium with the DR subregion. The DR series is encoded by the DRβ1 locus and the DRw52 and DRw53 alleles by the DRβ3 locus (Fig 5). The DQw3 is almost always associated with the DR4, DR9, DR5 and the DQw2 is almost always associated with the DR7, DR3. Similarly DQw1 is almost always associated with the DRw6, DR1, DR2 and DRw10 (Gregersen, 1989). Therefore, these patterns of linkage disequilebrium between DQ series, DR series and their DRW53, DRw52 associated genes (Fig. 6) reflect a characteristic relationship among these haplotypes. However these DQ / DR associations may differ in non-caucasian populations such as American Blacks (Hurley *et al.,* 1988). Polymorphism in the DQ subregion is present in both DQα and DQβ chain genes. The degree of variation in DQα and DQβ chains is less than that seen in DRβ chains in terms of the number of alleles involved and in the degree of variation at the particular residues. However, possible combinations of the variable DQα and DQβ chains will increase the DQ polymorphism further. Additionally combinations of α and β chain alleles will lead to an additional diversity that is characterising heterozygous individuals. The principle of this combinatorial diversity is the formation of hybrid DQ molecules through trans association of DQα and DQβ chains encoded in different haplotypes (Charron *et al.,* 1984).

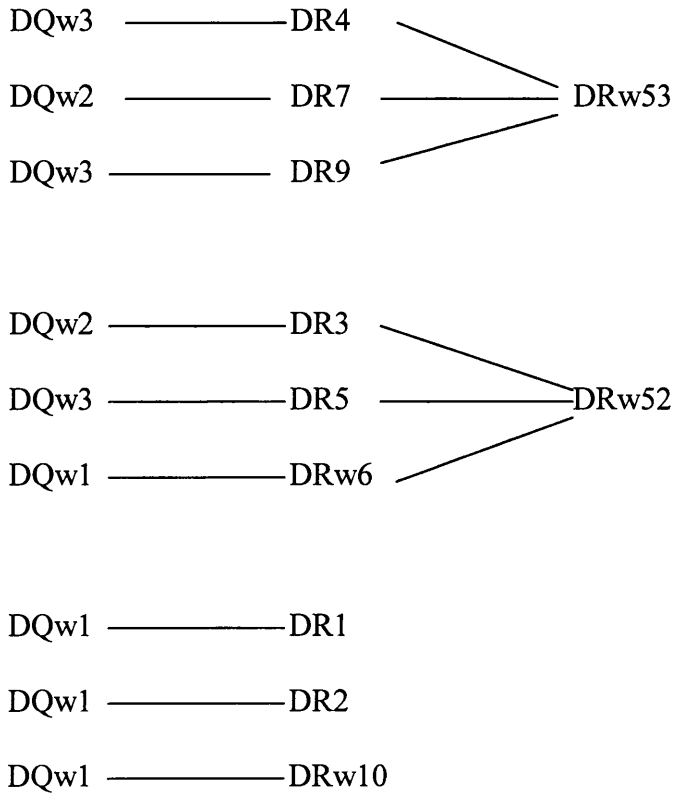


Fig 6. To show the patterns of linkage disequilebrium that is commonly found on HLA class II haplotypes in Caucasian populations (Gregersen, 1989).

DQB1 sequence variability in the upstream regulatory region of HLA class-II has been described as an additional mechanism of diversity. Twelve HLA DQB1 upstream regulatory region variants have been identified approximately 600bp immediately upstream of the first exon of the DQB1 gene (Reichstetter *et al.,* 1996). This study demonstrated a distribution of promoter sequence variability within DQB1 alleles coding region to be in strong linkage disequilebrium with exon 2 of the DQB1 in a Caucasian population. The DQB1 / QBP1 (DQB1 promoter) promoter haplotype presented a ratio of 75.9% for all DQB1*0501 positive haplotypes alleles. Between DQB1*0603 positive haplotypes, DQB1*0603/QBP1-6.3 was present at 96.4% and 95.2% of the DQB1*0604 positive haplotypes carried QBP16.2. Such studies indicate that these allelic polymorphisms may associate with differences in both transcription and affinity for DNA binding proteins. Functionally, such polymorphisms are related to the ability of HLA to bind and present peptides to T-cells. Thus, allelic polymorphism and promoter variability may contribute to the polymorphism of these heterodimeric molecules. Moreover, disease susceptibility may be influenced by variability in regulatory regions of genes (Haas *et al.,* 1995).

Many population-based studies have focused on HLA class II alleles previously, trying to identify association or predisposition to diseases. Basal cell carcinoma is a common skin cancer. In Swedish population its incidence has increased. A study by Emtestam *et al.,* (1996) demonstrated that DR1 frequency was the same in controls and patients but all the DR1 positive patients and controls carried the DQA1*01101 and DQB1*0501 alleles. This study showed that within HLA class II, there is a linkage between alleles coding regions but unfortunately they do not always contribute to aetiology of disease. However in Danish pregnant women it has been shown that HLA DR allogenotypes DR1/ DQ5 and/or DR3 are closely associated with predisposition in recurrent spontaneous abortions (Christiansen *et al.,* 1996).

In cutaneous malignant melanoma (CMM) the DQB1 *0301allele has been suspected to have a strong association with tumour progression in US patients according to

Lee *et al.,* (1994). Patients with the *0301 allele showed increased frequency of malignant disease considering stages of progression (56% CMM Vs 27% control; P=0.003). The *0301allele is associated with advanced disease and thicker primary tumours. Lee *et al.,* (1996) also show that *0301 allele carriers have an increased risk of developing recurrent CMM (49%) compared with stage-matched patients who lack the *0301allele (22%). In this study of Lee *et al.,* (1996) the HLA DQB1 *0301allele is reported as the most important allele associated with CMM. In a similar study by Bateman *et al.,* (1997) within the British population, the HLA DQB*0303 allele was found to be associated more with CMM patients than a control population (19.2% vs 5.8%; P=0.003; RR=3.9) where DQB1 *0301 was found to be associated with more advanced tumours and poorer prognosis. Thus in the British population *0301 and *0303 alleles may play a prognostic role in CMM where in the US population only the *0301allele has been shown to be a prognostic indicator. A similar study in an Italian population by Lombardi, (1998) revealed the association of DQB1 *0501allele with melanoma (patients 25% Vs 14.7% controls; P=0.038) where the *0301allele was found in similar numbers (62.8% patients Vs 54.9 % controls; P=0.136). Because DQB1 *0301 and DQB1 *0501are strongly associated with DRB1 *01 and DRB *11 both alleles DRB1 *01 and DRB *11 in the above study were found to be increased. In the British population (Bateman *et al.,* 1997) and in the US population (Lee *et al.,* 1996) such alleles show no association with melanoma. Such studies and many others within the literature, (examples as in Table 1) in different populations and diseases indicate the involvement of HLA molecules in onset and progression of diseases.

Table 1. To show examples of studies in different populations and diseases that the involvement of the HLA molecules was studied.

Disease	Author	Year	Population	Study conclusion
Cervical cancer	Gregoire, *et al.*	1994	African-American	Association
Cervical cancer	Helland, *et al.*	1994	Norwegian	Association
Cervical cancer	Nawa, *et al.*	1995	Japanese	Association
Cervical cancer	Odunsi, *et al.*	1995	S England	Association
Cervical cancer	Odunsi, *et al.*	1996	British	Association
Cervical cancer	Sanjeevi, *et al.*	1996	Swedish	Association
Cervical cancer	Montoya, *et al.*	1998	Spanish	Association
Cervical cancer	Helland, *et al.*	1998	Norwegian	Association
Cervical cancer	Ferrera, *et al.*	1999	Honduran	Association
Leukaemia	Dearden, *et al.*	1996	British	Association
Leukaemia	Taylor, *et al.*	1997	British	Association
Leukaemia	Manns, *et al.*	1998	African	Association
Celiac Disease	Martin, *et al.*	1995	British	Association
Ulcerative colitis	Reinsagen, *et al.*	1995	German	Association
Ulcerative colitis	Roussomoustakaki	1997	British	Association
Laryngeal papillomatosis	Aaltonen, *et al.*	1999	Finland	Association
Bowel Disease	Bouma, *et al.*	1996	Dutch	Association
Hepatitis C	Asti, *et al.*	1999	Italian	Contribution
Hepatitis C	Aikawa, *et al.*	1996	Japanese	Contribution
Rheumatoid Arthritis	Irene, *et al.*	1999	American	Association
Lung cancer	Hiraki, *et al.*	1999	Japanese	Association
T cell lymphoma	Jackow, *et al.*	1996	American	Association
Non Hodgkin's Lymphoma	Nathalang, *et al.*	1997	Japanese	Contribution
Pemphigus	Lee, *et al.*	1998	Korean	Association
Tuberculosis	Goldfeld, *et al.*	1998	Cambodian	Association

In the case of gastric cancer onset in the Caucasian population the HLA genes are believed to have an association. A resent study by Lee *et al.,* (1996) in Caucasian patients with gastric, colorectal and pancreatic adenocarcinoma revealed that the DQB1 *0301 allele is strongly associated with gastric adenocarcinoma 54% versus controls of 27%. After Bonferoni correction the data showed a P=0.003 by chi square test (odds ratio 3.2). No other HLA DQB1 allele and HLA DQA1, or TAP 2 alleles were found to be associated. The association of HLA DQB1, with Helicobacter *pylori* infection was investigated by Lee *et al.* (1996). Serological evidence for Helicobacter *pylori* in DQB1 *0301 positive patients with adenocarcinoma, compared with DQB1 *0301 negative patient population presented a lower frequency of DQB1 in positive 52% Vs negative 88% patient population (Fisher's Exact Test P=0.007). Such results lead to the conclusion that the DQB1 *0301 allele is more common in Caucasian patients with adenocarcinoma than controls and the mechanism linking the DQB1 *0301 allele with gastric adenocarcinoma is not likely through increased susceptibility to *Helicobacter pylori* infection. In the other types of cancer tested no association was found for any of the DQB1 alleles. However, Ohmori *et al.,* (1997) in the Japanese population revealed no statistical significance of any HLA DQB1 alleles or haplotypes in patients with gastric cancer ($P< 0.05$) compared with controls ($P< 0.05$). It is believed that in the Japanese population the environmental factors such as diet contribute more to gastric cancer than the genetic factor (Ohmori *et al.,* 1997). Such comparison of studies confirmed that gastric cancer is a multifactorial disease. Having such studies in mind, in this project DNA typing was performed using samples from West of Scotland-Glasgow population, looking for reproducible results such that of Lee *et al.,* (1996).

THE MHC CLASS III GENES

Many genes are located within the HLA class III including TNF, the HSP genes, complement factors and markers such as D6S273 (Fig 3). Among the HLA class III, TNF is a very important molecule involved in inflammatory responses. It is believed that it has a specific role in tumour pathology and more importantly in invasion of cancers. MHC class III region is the region where TNF genes exist coding for TNF-α. This region also includes the lymphotoxin α and β genes. The close relationship between TNF-α and lymphotoxin α was not known until 1984 when cloning of the cDNAs of Human TNF and lymphotoxin revealed that they are 30 % homologues at amino acid level (Gray *et al.,* 1984; Pennica *et al.,* 1984). TNF-α and lymphotoxin α bind to the same cell surface receptors and they are very similar in the spectra of their activities (Aggarwal *et al.,* 1985). The TNF locus is 12 Kb in length. The TNF locus is highly polymorphic. Within this locus five microsatellites have been identified, TNFa to TNFe (Nedospasov *et al.,* 1991). TNFa consists of fourteen alleles TNFa 1-14. TNFa is an inducible cytokine with a broad range of proinflammatory, catabolic and immunostimulatory actions. These include induction of IL-1, IL-6, IL-8, and TNFa itself, stimulation of prostaglandin E2, nitric oxide synthase and collagenase production, and increased B and T cell proliferation and immunoglobulin synthesis. TNF is produced mainly by activated macrophages but expression also occurs in T and B-lymphocytes, natural killer cells, and keratinocytes. TNF is involved in inflammation by enhancing the cellular migration of monocytes and macrophages (Beutler *et al.,* 1989). The gene encoding TNFa microsatellite lies within the MHC class III region, approximately 350Kb telomeric of the complement cluster and 320 Kb centromeric of the HLA-B locus as shown in Fig 7. The discovery of microsatellites and the location of TNF within the MHC has prompted much speculation about the role of TNF alleles in the aetiology of MHC-linked diseases, in particular those with an inflammatory or autoimmune component.

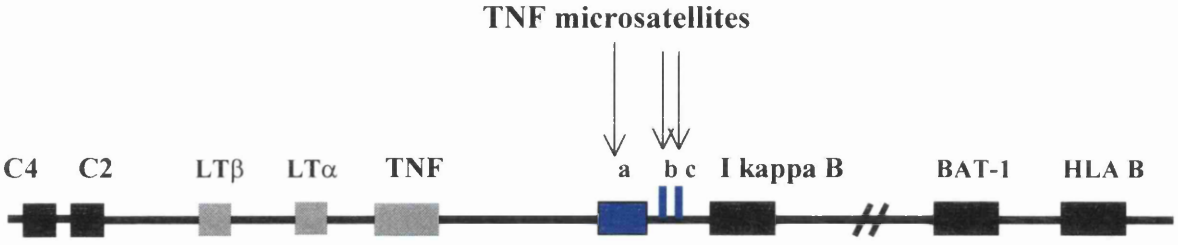


Fig 7. To present a schematic description of the TNF region.

The TNF gene locus, the lymphotoxin gene loci and microsatellite loci are shown in light blue colours. The map is not to scale.

In the case of *Helicobacter Pylori* infection and gastric cancer it is believed that TNF increases the production of gastrin due to TNF over-expression where an increase in acid secretion initiates ulceration. Chronic inflammation at the site may results in gastric cancer. The actual relation of *Helicobacter Pylori* and gastrin releasing hormone and TNF gene expression is not well known. A possible mechanism could be that the *Helicobacter Pylori* infection causes inflammation and increased regulation of TNF that results in up regulation of G-cell function resulting in increased release of gastrin, whereas development of chronic ulceration and inflammation will lead eventually to gastric cancer (Beales *et al.,* 1996). TNF genes and TNF microsatellites have been found to be involved in other diseases such as, coeliac disease (McManus *et al.,* 1995), colorectal cancer (Gallagher *et al.,* 1997), IDDM (Moghaddam *et al.,* 1997) and SLE (Tarasi *et al.,* 1997; Rood *et al.,* 2000).

In 1995, McManus *et al.,* reported that the TNFb3 microsatellite allele is significantly associated with coeliac disease (CD), which found in 86% of CD patients versus 24.5% of controls, with allele frequencies of 0.5392 and 0.1290, respectively ($P < 0.001$). Additionally the TNFa2 allele that was examined, presented frequency of 0.6122 in CD patients and 0.2627 in controls ($P < 0.001$), with phenotype of 87% for CD patients versus 50 % for controls. TNFb3 showed maximal level of linkage disequilebrium with HLA class II DQB1 *0201 in CD patients. However, while the HLA class II DQB1 *0201 / TNFa2 haplotype was strongly associated with CD, HLA class II DQB1 *0201was not significantly found in linkage disequilebrium with TNFa2, suggesting that TNFa2 is independently associated with CD. This association may have functional significance as TNFa2 has been correlated with high TNF production (McManus *et al.,* 1995).

In 1997, Gallagher *et al.,* reported strong association of TNFa2 allele with colorectal cancer in two different populations. In a Glasgow (British) population studied, the distribution of TNFa2 microsatellite out of the TNFa 1- 14 microsatellites that were examined, presented a phenotypic frequency of 62 (36.9 %) in colorectal cancer patients

(n=84) versus 37(20.33 %) of controls (n=91with significance of (P=0.0008) after Bonferoni's correction (Pc=0.0104). In an Essen (German) population that the study involved, the distribution of TNFa2 microsatellite out of the TNFa 1- 14 microsatellites that examined, presented phenotypic frequency of 47 (20.1 %) in colorectal cancer patients (n=47) versus 33(35.1%) of controls (n=117) with significance of (P=0.0066). This dual-population study shows clearly, that TNFa2 allele has a positive association with colorectal cancer (Gallagher *et al.,* 1997).

The hypothesis that TNF genes and / or TNF microsatellites, may be in linkage due to their position and function, with other loci on chromosome 6 (e.g. MHC), has trigger the curiosity of many research groups to proceed to further studies. In 1997, Moghaddam *et al.,* investigated the relationship between different microsatellites and HLA-DQ loci in insulin dependent diabetes mellitus (IDDM). IDDM is a genetically controlled autoimmune disease. It has been demonstrated that the HLA DQA1*0501-DQB1*0201/ DQA1*0301-DQB1* 0302 genotype is the most prevalent genotype in Caucasian IDDM patients (She *et al.,* 1996; Moghaddam *et al.*, 1997). Additionally it has been reported that DRB1*0403 protects against IDDM in Caucasian patients with genotype DR3- DQ2 / DR4 -DQ8 (Van der Auwera, 1995; Moghaddam *et al.*, 1997). To define additional markers for genetic susceptibility or protection in the HLA DQ high-risk genotype IDDM patients and controls (all having the HLA DQA1*0501-DQB1*0201/ DQA1*0301-DQB1* 0302 genotype), Moghaddam *et al.*, 1997, examined eight-microsatellite loci (HLA F, D6S273, TNFa D6S265, D6S1014, DQcar, TAP1, and D6S291) spanning the entire MHC region. The results presented no significant difference between the frequencies and homozygosity of the alleles. However, TNFa displayed a significant deference between diabetic patients and controls. For TNFa 47% of the control subjects and only 23% of the diabetic patients were homozygous (odds ratio 0.32; 95% CI 0.17-0.60; P <0.0003). Within the TNFa, marker allele *99 displayed a significant deference between diabetic patients and control subjects. Heterozygosity for allele *99 was observed in 62% of the patients and in 35% of the

controls with (P<0.0005). Linkage disequilibrium analyses revealed strong linkage disequilibrium of TNFa *99 and DRB1*0301 in both patients and controls but absence of TNFa *99 from other haplotypes (e.g. DQ8-DR4) in patients is associated with susceptibility to IDDM. The results suggested that two or more susceptibility loci in the MHC correlated with increase susceptibility to IDDM and that TNFa *99 / DRB1*0301could be an additional marker for susceptibility to IDDM (Moghaddam *et al.*, 1997).

Other studies such as that of Tarasi *et al.,* (1997) in a Greek SLE population suggested that TNF polymorphism may be related to renal complications of SLE patients but this relationship was closely linked between DRB1 *1501 and *1601 and *0701. Linkage disequilebrium described between DRB1 *1501, TNFa 11 and DR3 and TNFa2, b3, d2 but a significant association was not reported. A recent study by Rood, *et al.,* 2000, reported that TNF –308 and HLA-DRB1*0301 alleles contribute independently to susceptibility to SLE. The results presented TNF-308A or DRB1*0301 alleles to be more prevalent among SLE patients than control population with odd ratios of 0.5 and 4.4 respectively. The hypothesis that an association between TNF-308A and DRB1*0301 might cause DRB1*0301 to act as a confounder in the relationship between TNF –308A and SLE was tested. The results show that DRB1*0301 was significantly associated with TNF –308A ($P < 0.0001$). However, further analysis in that study revealed that TNF –308A and DRB1*0301alleles are independent susceptibility factors for SLE (Rood, *et al.,* 2000).

The identification of several allelic polymorphisms in very close physical linkage to the TNF locus has opened the way to a systematic study of associations between TNF alleles and the development of MHC linked diseases. Our study produced DQB1 allelic data from patients with gastric cancer and patients with Barrett's oesophagus that compared with a pre-existing TNFa microsatellite-allelic data from the same patients in order to discover any allelic relations.

8. MICROSATELLITES OVERVIEW

Microsatellites form a significant proportion of repetitive DNA sequences that widely incorporated in the human genome. Microsatellites are non-coding DNA sequences. Non-coding regions of DNA may play a functional role in the genome's function, either directly via a role in gene regulation (Hamada *et al.,* 1984; Koreth *et al.,* 1996) or indirectly as hot spots for recombination (Sligthon *et al.,* 1980; Koreth *et al.,* 1996). Non-coding regions could be located within genes or between genes and are called "introns". They are embedded in unique sequence and they can be amplified in vitro by the polymerase chain reaction (PCR). Tremendous genetic diversity has been allowed to develop in these regions. Much of this non-coding DNA consists of highly repetitive segments of DNA, consisting of several alterations of specific sequence known as "DNA-repeats". Microsatellites display considerable polymorphism due to variation in the number of "repeats" (Hearne *et al.,* 1992). Therefore they are ideal markers for constructing high-resolution genetic maps in order to identify susceptibility loci involved in genetic deceases.

DNA repeats can occur as a tandem array but not always. Such sequences are called variable number tandem repeats (VNTRs). One such class of sequences in humans consist of simple tandem repeat (STRs) often a dinucleotide sometimes a tri – tetra repeat consisting of **CA** (Adenosine-Cytosine) on one DNA strand and **GT** (Thymine-Guanine) on the other and / or **CT** (Cytosine- Thymine) on one DNA strand and **GA** (Guanine-Thymine) on the other. Such repeats of 2-5 nucleotide segments are known as microsatellite DNA and usually are repeat 15-30 times. A single pair of PCR oligonucleotide primers that surround such sequences can produce variable sized DNA fragments depending upon the number of repeats (Koreth *et al.,* 1996).

Such repetitive sequences have been classified as follows.

1. Satellite sequences: arrays with repeat sizes ranging from 5-100 bp organised in clusters up to 100Mb. These are located in heterochromatin near chromosomal centromers and telomers and are not variable in size within populations.
2. Minisatellites: arrays with repeat sizes of 15-70 bp, which range from 0.5 to 30 kb. Minisatellites are found in enchromatic regions of the genome and are highly variable in repeat size within populations
3. Minosatellite sequences: arrays with a repeat size 2-6 bp variables in size varying around 100bp.

The human genome contains large number of microsatellite repeats, each of which can exhibit a high degree of polymorphisms and are inherited with a Mendelian fashion, making them useful genetic markers. The human genome contains 50,000-100,000 interspersed microsatellite repeats of CA (Adenosine-Cytosine) occurring every 30-60Kb in enchromatic regions of DNA. Their function is largely unknown but it has been proposed that they may play a role in recombination and gene regulation (Martin *et al.,* 1998; Hearne *et al.,* 1992).

Within and flanking the MHC, approximately 50 microsatellites have been typed, most of which are CA repeats. Some of these were used in studies involving the MHC to map genes thought to be responsible for diseases, such as the haemochromatosis gene, which is located telomeric to HLA-A (Feder *et al.,* 1996). Recent laboratory findings suggested that microsatellites might be useful markers for mapping regions of disease (Martin *et al.,* 1998). A paradigm of some microsatellite markers across the HLA region can be seen in (Fig 3).

The possibility of the involvement of cytokines in the genetic predisposition to diseases has been proposed by a large variety of studies. Production of cytokines is genetically regulated in the sense that the capacity to respond to stimuli is determined by the genetic make up of individuals (Jacob *et al.,* 1993). Deregulated cytokine production

has shown to be implicated in various immune deficiencies, autoimmune and anti-inflammatory (Jacob *et al.,* 1993). For example, variation of TNF-α has been seen in human diseases such as autoimmune lupus nephritis (Jacob & McDevitt *et al.,* 1988), SLE (Jacob *et al.,* 1990) and in rodent models (Haranaka, *et al.,* 1984). In this frame the main idea was to consider HLA associated diseases where one HLA component is sufficiently well known and where the direct involvement of HLA pathophysiology of disease, is certain or highly probable. Various disease populations and control populations selectively matched for HLA markers that are known to be involved in a disease, and typed for microsatellites in order to detect additional susceptibility markers.

This area of application of microsatellite HLA typing is broad and the interest in HLA microsatellite is still increasing. Many HLA region polymorphism studies in different populations have taken place (Foissac & Cambon-Thomsen, 1998). The HLA DQ region has been characterised as important for such analysis because it presents high level of linkage disequilibrium with HLA DR region that often results in complex patterns of class II allelic associations. For example in coeliac disease trans complementation of HLA DQA1-DQB1 alleles suggest a specific role of the DQ heterodimer in disease susceptibility. In the HLA region recently a microsatellite marker located 1-1.5 Kb centromeric to HLA DQB1 has been characterised. This is the DQ CAR (Lin *et al.,* 1997). However, many other microsatellite markers have been described with HLA, too many to describe (Foissac & Cambon-Thomsen, 1998).

Instability in microsatellite repeats could cause genetic abnormalities that may lead to carcinogenesis. Microsatellite instability recently has been suggested to result in development of neoplasia. Microsatellite instability can be defined as the condition of novel microsatellite alleles in tumours. It is believed that mutations in the mismatch repair genes are responsible for development of some tumours, which exhibit microsatellite instability. A paradigm would be the discovery of microsatellite instability in hereditary nonpolyposis colon cancer (HNPCC) an autonomic dominant syndrome with

predisposition to colorectal and endometrial cancers (Lynch, *et al.,* 1991). The first clue came from the observation of a previously unrecognised phenomenon in HNPCC, new microsatellite alleles " T " DNA compared to non-neoplastic " N " DNA. In HNPCC microsatellite instability was discovered to be due to germline mutations in genes that encode the components of DNA proofreading complex (Kunkel, 1993). In HNPCC linkage to the marker D2S123 on chromosome 2p was reported and it was noted that instability of microsatellite repeat number was present in microsatellites through out the genome (Peltomaki *et al.,* 1993; Aaltonen *et al.,* 1993). Candidate genes in the region of linkage on chromosome 2p were screened and the hMSH2 gene was identified by sequence homology to the yeast MSH2 gene and its protein product was shown to be a DNA mismatch binding protein (Leach *et al.,* 1993; Fishel *et al.,* 1993). Additional mismatch repair genes where shown to be involved in HNPCC such as hMLH1, hPMS1 and hPMS2 that are homologues to bacterial *mut*-HLS (Bronner *et al.,* 1994; Nicolaides *et al.,* 1994). Microsatellite instability has been identified in a high range of tumours such as sporadic colon carcinomas as well as endometrial carcinoma and gastric cancer with multiple replication errors as in HNPCC cases (Woostrer *et al.,* 1994; Peltomaki *et al.,* 1993). In contrast with other sporadic tumours such as lung, breast, testis, CNS tumours and soft tissue sarcomas, microsatellite instability is present but has been described as a rare event, only affecting a single microsatellite loci (Woostrer *et al.,* 1994; Peltomaki *et al.,* 1993).

The contribution of such genomic instability to carcinogenesis is not clear as yet. What is unclear is whether a mutation targeting specific sequence repeats or that the genomic instability has general effects, non-specifically activating oncogenes and / or inactivating tumour suppressor genes. Markowits *et al.,* (1995) reported that TGF-β receptor gene (a tumour suppressor gene) is inactivated specifically in colon cancer cell lines favours the former feasibility.

HEAT SHOCK PROTEIN 70 FAMILY AND (HSP-70-2) MARKER

Heat shock proteins (HSPs) are present in both prokaryotic and eukaryotic cells. Their highly complex structure suggests that they play a role in intracellular processes. Heat shock proteins are synthesised in response to stress. Rapid induction of heat shock proteins accompanies, a decrease in transcription and translation of other genes and gene products. HSPs are present in the cytosol, mitochondria, endoplasmic reticulum and nucleus. They have a relatively long half-life of 48 hours in human epidermoid cells. The complete function of these proteins is not clear as yet. There is substantial evidence linking heat shock proteins with a protective mechanism during and following cellular stress. The protection is probably mediated by the HSP's capacity to function as molecular chaperones to prevent inappropriate protein aggregation and to mediate transport of immune proteins to the target organelles for final packaging, degradation or repair (Kiang *et al.,* 1998). Furthermore, there is a close relationship between heat shock protein expresion and malignant transformation in mammalian cells (Harrison *et al.,* 1987).

The locus encoding for the major heat shock protein (HSP-70) is located between the complement and the TNF genes (Fig 8). The HSP 70 loci are 12 Kb apart and lie 92 Kb telomeric of C2 genes (Milner & Campbell, 1990). HSP 70 proteins have a protective role during and after cellular stress (Sargent *et al.,* 1989). Nucleotide sequence analysis of the two introless genes HSP 70-1 and HSP 70-2 has shown that these genes encode an identical product of 641 amino acids long (Milner & Campbell, 1990; Hunt & Morimoto, 1985). A third introless gene HSP 70-Hom exists in the HSP70 region and located 4 Kb telomeric to HSP 70-1 gene. This encodes for a basic protein of 641 amino acids long, which have be found to have 90% sequence similarly with HSP 70-1 (Milner & Campbell, 1990). The HSP-Hom appears to be constantly expressed but the levels of mRNA are not increased following heat shock (Milner & Campbell, 1990). The fact that two closely linked loci both express an identical protein is surprising. A possible explanation could be that the range of stress factors that induce HSP 70 expresion is too high for the promotor

sequence of a single gene to recognise them all. Thus, two genes HSP70-1 and HSP70-2 are required to provide adequate response. Expression of HSP 70 is related to endothelial cell activation through adhesion molecules such as ICAM-1, VCAM-1 and release of IL-8, which has been found being increased in alograft presentation studies and related endothelial injury studies (Eberl *et al.,* 1999). However, expression of HSP 70 is related to age according to Rao *et al.,* (1999). Rao *et al.,* (1999) demonstrated that there is an age related reduced expresion of HSP 70 in human peripheral lymphocytes. Low expresion of HSPs may correlate with decreased resistance to infections and increased mortality in the elderly. It is noteworthy in this respect, that lymphocyte activity induced by interleukins and mitogens also induces the synthesis of HSPs at the transcriptional and translational level. Moreover, HSPs associated with processing of protein antigens that associated with human diseases such as malaria and leishmaniasis for example (Rao *et al.,* 1999). In many bacterial and parasitic infections the HSP 70s are the immunodominant antigens of the infecting organisms (Kiang *et al.,* 1998). HSPs are present only intracellularly. How the T-cells recognise the bacterial or parasitic HSPs is not clear yet. It is believed that the bacterial produced protein GroEL is secreted or present on the surface membrane and allows T cells to bind to the GroEL antigen. Another possibility is that cytolysis of infected cells releases pathogenic HSPs to the extracellular space where they are detected by host immune cells. Macrophages respond to infection by releasing cytokines, oxygen free radicals and nitric oxide, which are involved in killing the infected cells (Snyder *et al.,* 1992). On the other hand the synthesis of HSPs in macrophages increased by the release of cytokines, oxygen free radicals and nitric oxide (Matnney *et al.,* 1992). However, increase in HSPs in macrophages in turn inhibits cytokines, oxygen free radicals and nitric oxide production leading to ineffective immune responses (Shigetada *et al.,* 1996). Thus, Increase expresion of HSPs may not benefit the host.

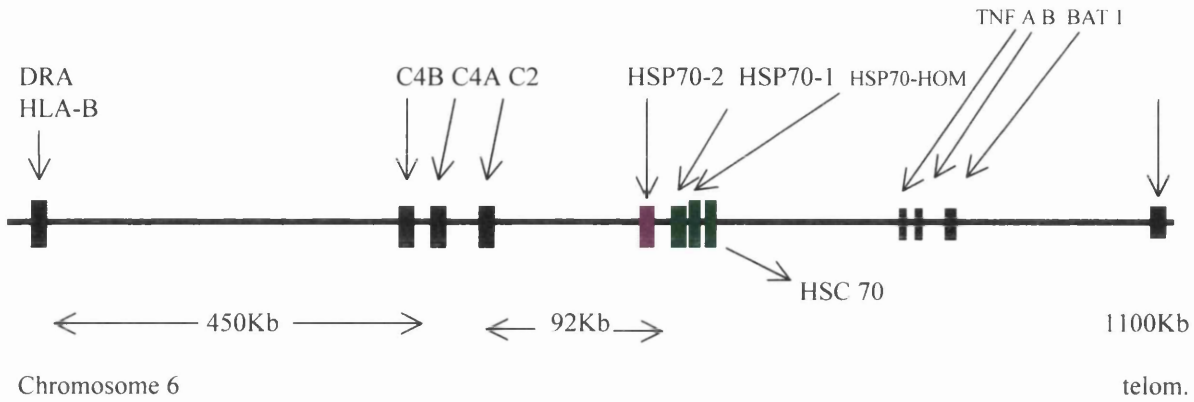


Fig 8. Molecular map of the MHC class III region, showing the position of the HSP 70 loci (green colour) in relation to other genes identified in this region. HSP 70 loci lie 92 Kb telomeric to the C2 gene. HSP 70-1 and the locus of interest HSP 70-2 (violet colour) are 12Kb apart. The position of the cognate gene HSC 70 is also shown. The map is not to scale.

HSP 70 is involved in bacterial infections as mentioned previously. Surface-associated HSP 70 mediates *Helicobacter Pylori* attachment to epithelial cells. The increase expresion of heat shock proteins following acid shock correlates with association with inflammation of gastric mucosa (Hoffman & Gurdano, 1999). Thus, heat shock proteins may play an important role in the colonisation of bacteria in the stomach and mucosal infection and promoting inflammation (Hoffman & Gurdano, 1999). This finding is a very important reason that has driven us to investigate the HSP 70 in this study. Also liberated HSPs may play a prominent role in promoting inflammation since it has been demonstrated that HSPs induce the production of INF-γ and TNF- α in mouse gastric tissue infected by *Helicobacter Pylori* (Hoffman & Gurdano, 1999). HSP 70 plays an important role in cytoprotection against TNF mediated cytotoxicity (Nishimura *et al.,* 1997). HSP 70 acts as protector against macrophage TNF induced cell death (Jaattela, 1999). This mechanism has been demonstrated previously in macrophages infected by bacteria such as *Salmonella* (Nishimura *et al.,* 1997). Although in other infections from protozoa such as *Leismania donovani* TNF plays an integral role in host response (Salotra *et al.,* 1995). TNF is essential for expression of cytotoxic levels of nitric oxide, which mediates intracellular killing of *Leismania donovani.* Furthermore TNF may itself produce proteins that protect cells from TNF mediated cytotolysis essential in macrophage survival (Wong *et al.,* 1989). However, induction of heat shock proteins by TNF has been demonstrated only in bacteria (Nishimura *et al.,* 1997).

Studies such that of Salotra *et al.,* (1995) and Ahn *et al.,* (1998) have demonstrated the role of HSP 70 in apoptosis. Heat shock proteins are involved in cell death. HSP 70 induces infiltrate of T-cells, macrophages and dendritic cells into tumours as well as Th1 cytokine expresion of IFNγ, TNFa and IL-12 where it enhances immunogenisity via a T-cell mediated mechanism. HSP 70 protection is both tumour and cell specific. HSP 70 acts as a chaperone and is involved in direct chaperoning into dendritic cells (Todryk *et al.,* 1999). Mutations of P53 gene are known to result in the generation of mutant proteins and

in tumourogenesis. HSP 70 in most cases binds mutant P53 proteins and presents them to leucocytes for elimination (Fourier *et al.,* 1997). Induction of heat shock proteins in many tumour cells renders them resistant to cytotoxicity mediated by TNF (Salotra *et al.,* 1995; Kusher *et al.,* 1990). Wei *et al.*, (1995) showed that HSP 70 gene overexpression in tumour cells inhibits cell proliferation. The induction of apoptosis that takes place suggests that HSP 70 is required for tumour cell proliferation and survival. Ahn et *al.,* (1998) demonstrated resistance to apoptosis by utilising a prostaglandin and c-myc mRNA expresion model. Decrease in prostaglandin (known to act as anti-proliferative) was related to the amount of c-myc expresion. Induction of c-myc regulated the elevation of HSP 70, resulting in decrease of intracellular apoptosis. Therefore tumour immunogenisity is determined by the mechanism of cell death via HSP 70s expresion. Research groups are now focusing on the utilisation of gene transfer to tumour cells in order to stimulate an anti-tumour immune response (Melcher *et al.,* 1998).

Several genes with anti apoptotic activity have been determined in studies such that of Mussarella *et al.,* (1998) in an avian blastodermal cell model derived from fertile chicken eggs. In that study genes including the bcl-2, bcl-$x_{L,}$ HSP 70, grp 78 and glutathionine S-transferase were expressed as early as in stage 1 in embryo of the newly oviposide egg. Significant levels of HSP 70 mRNA found when such genes were expressed. However the whole mechanism of induction of resistance to apoptosis is not clear yet. Many other proteins are probably involved. One example is the BAG-1 (Takayama *et al.,* 1998). Human BAG-1 proteins have 3 isoforms BAG-1, BAG-1M and BAG-1L. BAG-1 proteins have the ability to bind to HSP 70 and HSC 70, a HSP 70 cognate gene (Fig 8), which is constitutively expressed and shares 82 % similarity in its amino acid sequence with HSP 70 (Dworniczak & Mirault, 1987). The BAG-1 protein has been identified as a novel regulator of apoptosis. Its ability to bind to Bcl-2 results in increased levels of BAG-1. This has been seen in correlation with increased levels of Bcl-2 suppressing apoptosis via CD95 (fas/apo-1) in tumour cell lines from leukaemia, breast

cancer, prostate cancer and colon cancer that examined by (Takayama *et al.,* 1998). IL-2 and IL-3, up regulate the expresion of BAG-1 significantly for cell survival and enhances independent cell growth (Adachi *et al.,* 1996). In gastric cancer, the complex HSP 70 – Bcl 2 probably promotes cells survival where BAG-1 accelerates cell motility (Takayama *et al.,* 1998). Naishiro *et al.,* (1999), provided evidence that gene transfer mediated the over-expression of BAG-1. The result of this gene transfer was a remarkable increase in the motility of human gastric cancer cells but without any alterations in cell morphology or adhesion capacity. Heat shock proteins are directly expressed in human gastrointestinal cancers. HSP 70 and HSP 27 mRNA levels found to be differentially expressed in gastrointestinal cancers (Ehrenfield *et al.,* 1995). Similarly the results in pancreatic cancer and colon cancer in the same study suggested that deferential expresion of HSP 70 occurs but not as strongly as in gastric cancers. In other cases such that of squamus cell carcinoma of the oesophagus, the expresion of HSP 70 and HSP 27 was found to be frequently reduced, suggesting that HSP 70 and HSP 27 are independent prognostic factors (Kawanishi *et al.,* 1998).

The possibility that HSP 70 may be involved in various aspect of the immune system has risen. The specific location of HSP70 gene complex between the classical transplantation antigens and the TNF locus makes the nature of these proteins and genes obviously vital in different diseases involving bacterial infections as described above and cancer. Additionally, proof exists that HSP 70 is involved in bacterial colonisation, infection and inhibition of apoptotic cascades in cancers including gastrointestinal cancers. Taken into consideration all those facts, it was deemed important to investigate the role of HSP 70 locus in this project. The primer sequences for the HSP 70-2 microsatellite analysis that utilised in this study are described in the method and materials section.

The D6S273 MICROSATELLITE MARKER

The microsatellite marker D6S273 has a range of 126-142 bp in size. It is located between the HSP70 locus, BAT 2 and the TNF locus within the MHC III (Fig 3). The MHC class III region remains among the most gene- rich regions of the human genome with on average, one gene per 10 Kb of DNA. Because of its location near HSP-70 that is known to be involved in carcinogenesis and inflammation, and near TNF another factor that is involved in carcinogenesis and regulation of inflammation, and also because of its positive involvement with diseases that described below, D6S273 was the best possible site to investigate next.

A 30Kb segment of the MHC class III region lying between the heat shock protein HSP-70 and TNF genes has been recently sequenced by Ribas *et al.*, (1999). The sequencing revealed the localization of seven genes whose precise position and order is cen-G7-G6-G6A-G6B-G6C-G6D-G6E-tel. These genes belong to the Ly-6 superfamily. It is believed that the Ly-6 superfamily of genes, are involved in leukocyte maturation. The microsatellite marker D6S273 was contained within this sequence. In different population studies, D6S273 microsatellite alleles and TNFa microsatellite alleles presented a variation in their distribution. This variability of expression may result in the variability of onset of diseases including cancer. In studies such as that of Gubric *et al.*, (1999) in a Croatian population this kind of variation-irregularity in specific microsatellite alleles in different populations were confirmed, compared with studies in other populations such as in Dutch, Italian, Greek, Basques and Danish. Then most frequent alleles at D6S273 locus were D6S273 134bp and 136bp while at TNF locus two most frequent alleles were TNFa 117bp and 99bp. In that study it was concluded that the irregularity in distribution of microsatellite alleles in different populations with the predominance of two or three alleles on these two investigated microsatellite loci was present.

Different studies have observed possible association of D6S273 microsatellite alleles with diseases. Marker D6S273 has now been reported to be strongly associated

with autoimmune diseases such as Ankylosing Spondylitis (AS), as reported by Brown *et al.,* (1998) in the British population and probably it is in linkage with other genes within the HLA class III region, such as the HSP 70 that is found not to be independently associated with (AS) by Fraile *et al.,* (1998).

Another example that indicates the association of D6S273 marker with disease is that of IDDM1. IDDM1 is a genetically controlled disease resulting from the destruction of β-cells in the pancreas. D6S273 microsatellite alleles are found to contribute to the genetic risk, especially the alleles 136 and 140. However a recombination linkage between DQ and TNFa was reported as an additional contributory factor in IDDM1 (Moghaddam *et al.*, (1998). In contrast, the HLA-DR and TNFβ markers have been reported by Haines *et al.*, (1998) to have strong genetic linkage with D6S273 marker and the onset of Multiple Sclerosis (MS). In other diseases such that of Rheumatoid arthritis (RA) D6S273 is now believed to have an association. Rheumatoid arthritis (RA) is a chronic inflammatory disease that is associated with the HLA-DRB1 genes. A resent study by Signal, D.P. (1998) revealed that two D6S273 microsatellite alleles 132 and 138 were significantly associated in Canadian patients with RA. Such studies suggest that D6S273 has not an independent association with diseases but probably are in strong linkage with other markers.

D6S273 marker now believed to be linked with some types of cancer. However not many studies have utilised this marker to investigate cancer and the literature lacks a significant amount of information regarding the cancer issue. One example of the few cancer studies that utilized the D6S273 marker is that of Mazurenko *et al.*, (1999) regarding cervical cancer where demonstrated that deletion of alleles in chromosome 6 leads to LOH and microsatellite instability MIN. In the study curried out by Mazurenko *et al.*, (1999) it was reported that the highest LOH was observed in the HLA region within the markers D6S273 and D6S276 where MIN was found in 3 out of 62 cases. Such results suggest that several regions in chromosome 6 might harbour potential tumour suppressor genes important in cervical cancer.

Taken together such studies and the lack of extensive information in literature regarding D6S273 and cancer, good reasons existed to lead us in the investigation of the distribution of D6S273 alleles in a West of Scotland (Glasgow) population and tried to estimate association of any of the D6S273 microsatellite alleles with gastric and oesophageal cancer.

AIMS OF THE PROJECT

The genetic control of inflammation must be an important factor in pre-malignant cases of Barrett's oesophagus and gastric cancer. The location and expresion of certain genetic loci such as DQβ1, HSP 70 and polymorphic sites such as TNFa and D6S273 within chromosome 6 suggests their importance in this control. The fact that TNF locus has already been implicated in malignant progression through its ability to promote the invasive properties of tumour cells in gastrointestinal cancers, further fuels the hypothesis. Taking into account the information already known linking genotype expresion of such markers and disease several aims were drawn up.

First to carry out a thorough allelotype and genotype analysis of the sites around TNF locus on human chromosome 6 in a Barrett's oesophagus population and in a gastric cancer population. In doing so, to optimise the reaction conditions for the detection of the alleles was necessary.

Second to determine whether the normal populations present similarities or differences to pre-malignant and malignant populations where certain alleles may present overexpression.

Third to compare the data obtained from the loci investigated in this study with that previously collected within the group regarding TNFa in Barrett's oesophagus and gastric cancer. This would allow the detection of any differences and / or similarities between the populations to be noted, with a possible suggestion as to whether genotype expresion patterns were a feature of these pre-malignant and malignant conditions.

Fourth to attempt to draw conclusions about the role of TNF, either as an independent factor or in conjunction with the other three loci that were examined.

There are no previous attempts at this project but a small number of similar studies in different diseases including some other various types of cancer, have utilised similar and / or the same genetic markers concluding to positive results. The whole experimental

process was then carried out within a laboratory at the Lister Department of Surgery at the North Glasgow Hospitals University Trust.

MATERIALS & METHODS

MATERIALS AND METHODS

DNA - EXTRACTION

Genomic DNA was used from a panel of stock samples that already existed in the laboratory. Extraction of this genomic DNA, had previously been carried out by others using the Dynal® DNA extraction Kit (Tissue Typing-Dynal SSP, Technical Handbook, First edition, 1996) from whole blood lymphocytes, from malignant Barrett's oesophagus, gastric cancer and normal populations whereas the benign population DNA was extracted from a panel of histological materials (Slides) using the method as described by (Wright D.K., Manos M. M. "Sample preparation from paraffin-embedded tissues" In Innis, M., Gelfand, D. H., Sninsky J. J., White, T., eds. PCR Protocols: "A guide to method and applications". San Diego academic press, 1990; 153-158). The tumor location of the gastric cancer samples obtained was various, 11 were proximal gastric, 3 distal 1/3 oesophagus, 33 antrum, 16 corpus of stomach, 5 whole stomach and 2 gastro-jejeunostomy stoma.

The concentration of samples ranged from 5 ng /μl to 10 ng /μl for the benign DNA samples from patients with Barrett's oesophagus, whereas the malignant DNA samples from patients with Barrett's oesophagus, and DNA samples from patients with gastric cancer ranged between 50 ng /μl to 100 ng /μl. The normal DNA needed to be diluted to 50 ng /μl to be effective.

PART 1. DQβ1 –Typing

Study subjects

DNA samples from 101 disease free Caucasian unrelated subjects, from 51 Caucasian unrelated patients with Barrett's adenocarcinoma oesophagus, from 37 Caucasian unrelated patients with benign Barrett's oesophagus and from 44 Caucasian unrelated patients with gastric adenocarcinoma were investigated. The patient and control population originated from the West of Scotland. The control population data was obtained from the west of Scotland tissue typing laboratory, Glasgow Royal Infirmary.

Polymerase chain reaction (PCR)

Dynal *All*set™ SSP DQ "Low resolution sets" were used. The protocol is based on (Aldener-Canava & Olerup, 1994; Olerup, *et al.,* 1993). Each PCR-SSP 8-tube reaction per person mixture was consisted of 10μl of each genomic DNA sample (1μl per reaction), 1×Advanced Biotechnologies PCR buffer (200mM $(NH_4)_2$ SO_4, 750 mM Tris-HCl, pH (8.8) at 25 °C, 0.1% (v/v) Tween® 20), 1.5 mM $MgCl_2$, 200μM each of (dATP, dCTP, dGTP and dTTP), 0.5 units / reaction Ampli -Taq ™ DNA polymerase (Advanced Biotechnologies 5 units/μl), 10 % (v/v) glycerol, and Cresol Red, sodium salt (100 μg/ml) resulting to final volume of 100μl DNA-PCR solution-Ampli-Taq mixture. The PCR reaction micro-tubes and master mix tubes were always kept on ice during preparation. Ten microlitre of the DNA-PCR solution-Ampli-Taq mixture was dispensed into each of 8-microtube set (Fig. 9), pre-coated with the allele and group specific primer mixes and control primers matching non-allelic sequences. The allelic and serological relationship of DQβ1 is shown on (Table 2).

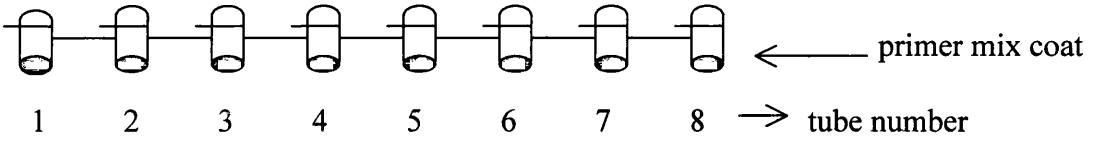


Fig 9. Tray lay out.

The tubes are pre-coated with the allele and group specific primer mixes and control primers matching non-allelic sequences. The coloured shaded areas represent the coated portion of the tubes.

Table 2. To show the allelic and serological relationship of DQB1 alleles, (Dynal *All*set™) SSP "DQ low resolution"

Tube number	Product size bp	Alleles	DQ Serology
1	220	DQB1 *0501 - DQB1 *0504	5
2	220	DQB1 *06011-DQB1*0613	6
3	205	DQB1 *0201- DQB1*0203	2
4	130,145	DQB1*0201, DQB1*0202, DQB1*0302, DQB1*0301-DQB1*0307, DQB1*0308	2, 8, 3
5	125	DQB1 *0301, DQB1*0304	7
6	125,140	DQB1 *0302, DQB1 *03032, DQB1 *0305- DQB1 *0307	8, 9, 3
7	125	DQB1*0203, DQB1*0301 DQB1 *03032, DQB1*0306	2, 7, 9, 3
8	130	DQB1 *0401, DQB1*0402	4

Adopted from the Dynal *All*set™ " DQ low resolution" literature.

PCR Cycling parameters

PCR amplification was carried out in a PTC-225 DNA Engine, Tetrad, (Version 0.3), 96-well plate thermal cycler. After an initial denaturation at 94 °C for 2 minutes the DNAs were amplified by 30 amplification cycles. The first 10 cycles consisted of denaturation at 94 °C for 10 seconds and a combined annealing and extension step at 65 °C for 60 seconds. The following 20 cycles consisted of denaturation at 94 °C for 10 seconds, annealing at 61 °C for 50 seconds and an extension at 72 °C for 30 seconds.

Visualisation of amplifications

The visualisation of the amplified DNA was performed by agarose gel electrophoresis. The whole PCR reaction products were directly electrophorised on 2 % agarose (SEA KEM LE™) or Ultra Pure agarose (GIBCO™) pre-stained with ethidium bromide (0.25μg/ml). The gels electrophorised for 20 minutes at 150 mAmps/cm in 1×TBE buffer solution consisted of (34 mM Tris-base, 34 mM boric acid, 4mM EDTA, pH 8.0) and visualised under ultra violet illumination. The PCR product obtained was dependent to the specific primer amplification taken place each time and the control PCR product was a standard of 425 base pair of the human growth factor as indicated in the manual of the Dynal Allset™ SSP set (October 1998). The ϕx174-RF DNA / Hαe III sequencing ladder (GIBO™. BRL.UK) or the hyperLadder-I™ (Bioline Ltd) was used to determine the fragments length.

PART 2. HSP 70-2 restriction fragment length polymorphism marker

Study subjects

DNA samples from 111 disease free Caucasian unrelated subjects, from 30 Caucasian unrelated patients with Barrett's adenocarcinoma oesophagus, from 26 Caucasian unrelated patients with benign Barrett's oesophagus and from 42 Caucasian unrelated patients with gastric adenocarcinoma were investigated. The patient and control population was originated from the West of Scotland.

Polymerase chain reaction (PCR)

In order to explore the possibility that HSP 70-2 gene locus may be involved in determining the susceptibility to Barrett's oesophagus and gastric carcinoma, the HSP 70-2 restriction fragment length polymorphism (RFLPs) was investigated by PCR. The coding sequence of the HSP 70-2 gene was amplified, using sequence specific oligonucleotide primers and considering the polymorphic Pst-I restriction site at position 1267 of these alleles. (Nucleotide 1267 of structural HSP 70-2 gene) The 5'-primer: 5'- CAT CGA CTT CTA CAC GT CCA -3' was used in combination with the 3'-primer: 5'- CAA AGT CCT TGA GTC CAA AC -3' (MWG Biotech AG, UK). The PCR reaction mixture consisted of 50 ng/μl genomic DNA, 200μM each of (dATP, dCTP, dGTP and dTTP), 2 mM $MgCl_2$, 1×Advanced Biotechnologies PCR buffer (200mM$(NH_4)_2$ SO_4, 750 mM Tris-HCl, pH (8.8) at 25 °C, 0.1% (v/v) Tween® 20), 1mM of each primer, 0.5 units Ampli-Taq™ DNA polymerase (Advanced Biotechnologies 5 units/μl), and two drops of liquid paraffin, in a total of 25 μl reaction volume.

PCR Cycling parameters

PCR amplifications were carried out in a Biometra Uno-thermoblock™ thermal cycler (Biometra™ USA) that did not have a heated lid. Amplification was accomplished by an initial denaturation at 94 °C for 5 minutes, followed by 30-35 cycles (depending to Sample origin and concentration) each consisted of further denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds, extension at 72 °C for 30 seconds and a final extension at 72 °C for 5 minutes.

Visualisation of amplifications

The visualisation of the amplified DNA was performed by agarose gel electrophoresis. Five microlitre of PCR reaction products were mixed with 5µl orange-G and electrophorised on 2 % Ultra Pure agarose (GIBCO™) pre-stained with ethidium bromide (0.25µg/ml). The gels electrophorised for 40 minutes at 80 mAmps/cm in 1×TBE buffer solution consisted of (34 mM Tris-base, 34 mM boric acid, 4mM EDTA, pH 8.0) and visualised under ultra violet illumination. The ϕx174-RF DNA / Hαe III sequencing ladder (GIBCO™. BRL UK) or the hyperLadder-I™ (Bioline Ltd) was used to determine the fragments length.

PCR -product digestion (RFLPs)

To assess the polymorphism (RFLP) of the HSP 70-2 at position 1267 the corresponding PCR products were digested with Pst-1 restriction digest enzyme 5'-CTGCA -3' and 3'- GACGTC -5' (Stratagene, USA). The digestion mixture were consisted of 1×Universal buffer (1 M koAc, 250 mM Tris acetate, pH 7.6, 100 mM MgoAc, 5 mM B-mercaptoethanol, 100 µg / ml BSA), 5 units of Pst-I restriction digest enzyme, sterile H_2O and 10µl of PCR product template. The mixture was incubated for 3 hours in order the digestion to take place.

Visualisation of RFLP products

The visualisation of the amplified DNA was performed by agarose gel electrophoresis. The PCR digested products were mixed with 5μl orange-G and electrophorised on 2 % Ultra Pure agarose (GIBCO™) pre-stained with ethidium bromide (0.25μg/ml). The gels electrophorised for 40 minutes at 80 mAmps/cm in 1×TBE buffer solution consisted of (34 mM Tris-base, 34 mM boric acid, 4mM EDTA, pH 8.0) and visualised under ultra violet illumination. The ϕx174-RF DNA / Hαe III sequencing ladder (GIBCO™BRL.UK) or the hyperLadder-I™ (Bioline Ltd) was used to determine the (RFLP) fragments length of (1117 bp) and (936 bp).

PART 3. D6S273 restriction polymorphism microsatellite marker

Study subjects

DNA samples from 105 disease free Caucasian unrelated subjects, from 32 Caucasian unrelated patients with Barrett's adenocarcinoma oesophagus, from 13 Caucasian unrelated patients with benign Barrett's oesophagus and from 37 Caucasian unrelated patients with gastric adenocarcinoma were investigated. The patient and control population originated from the West of Scotland.

Polymerase chain reaction (PCR)

In order to explore the possibility that D6S273 marker may be involved in determining the susceptibility to Barrett's oesophagus and gastric carcinoma, amplification of microsatellite locus D6S273 was performed in a total reaction volume of 10µl containing 50 ng of genomic DNA. The PCR reaction mixture also consisted of 1×Bioline ™ reaction buffer (160mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8 at 25 °C), 0.1 % Tween-20), 2 mM $MgCl_2$, 0.5 units Biotaq™ DNA polymerase(Bioline, Ltd, UK), 0.5µM of each primer, 200µM of each (dATP, GTP, TTP), and 1:10 of dCTP. The sequences of the D6S273 primers used for amplification were D6S273-1= 5'- GCA ACTT TTC TGT CAA TCCA -3' and D6S273 –2 = 5'- AC CAA ACT TCA AAT TTT CGG -3' (MWG Biotech AG, UK). The primer D6S273-1 was 5'-end labelled with [r^{32} P] d-CTP (370 MBq / ml-10 Ci / ml) purchased from Amersam Pharmacia biotech Ltd, UK.

PCR Cycling parameters

PCR amplifications were carried out in a Biometra Uno-thermoblock™ thermal cycler (Biometra™ USA) without heated lid. Amplification was accomplished by initial denaturation at 94 °C for 5 minutes, followed by 34 cycles each consisted of further denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds, extension at 72 °C for 30 seconds and a final extension at 72 °C for 30 seconds.

Preparation of 6% polyacrylamide gel

The 6% polyacrylamide gels were prepared by mixing 15 ml of 40% acrylamide solution with 20 ml of 5×TBE and with 7 M Urea. The final volume of 100 ml was adjusted with the addition of distilled deionised H_2O. The mixture was let to dissolve for about 1 hour. Finally 50µl of TEMED ($C_6H_{16}N_2$) and 750 µl of 10% ammonium persulphate was added for the polymerisation to take place. The solution was poured between a set of glass plates, separated with 1mm plastic spacers using a 50 ml syringe, where a sequencing comb was inserted to form a well. The gel was always left overnight in order the polymerisation to take place.

Visualisation of amplifications

The PCR amplified DNA was analysed following electrophoresis on a 6% denaturing polyacrylamide gel. Prior of loading the 6% polyacrylamide gel with the amplified DNA, 4 µl of denaturing enzyme formamide (GIBCO™BRL.UK) was added in each of the PCR amplified products and then placed in a Biometra Uno-thermoblock™ thermal cycler (Biometra™ USA) for 10 minutes at 80 °C for the denaturation to take place. The gels electrophorised for 3 hours at 75 mAmps/cm in 1×TBE buffer solution consisted of (34 mM Tris-base, 34 mM boric acid, 4 mM EDTA, pH 8.0). The polyacrylamide-sequencing gels were dried and autoradiographed overnight using

radiographic intensifying screens. The D6S273 alleles were identified by the size of the amplified fragments (126 bp, 128 bp, 130 bp, 132 bp, 134 bp, 136 bp, 138 bp) on a lightbox.

Optimisation of the protocols for determining genotype at the three loci

Attempts were made to optimise the PCR reactions and gel conditions of the individual three loci. For the optimisation of PCR reactions standard cell line DNA (Bristol, UK) was utilised. The optimisation of the PCR reactions was performed following some simple steps. The annealing temperature was first calculated by taking into consideration the melting temperature (Tm) of the primers from the manufactures documentation. After an initial PCR the annealing temperature was adjusted if it was necessary. An $MgCl_2$ titration was then performed in order to identify the best possible concentration of $MgCl_2$ that the certain PCR would produce appropriate results. This was varied from 0.5 mM to 2.0 mM in increments of 0.5 mM. The primer concentration was titrated in some instances. This was varied from 0.5μM to 1.5μM in increments of 0.5μM. A touchdown PCR program of cycling was tried as an alternative to the original decided. Sometimes the extension time was needed to adjust in order to avoid miss-amplifications that may interfere with the expected fragments. Although there were situations, in which Taq polymerase was tested from Ampli -Taq ™ DNA polymerase (Advanced Biotechnologies, UK), Biotaq™ DNA Polymerase (Bioline Ltd, UK) and Primezyme Taq™ in order to identify the most appropriate for the specific PCR to work with. In all cases the enzyme was supplied with a PCR Buffer and Mg Cl_2 buffer. Both were tried during the optimisation process. All the PCR reactions in this investigation were standardised in the same way. Genotype determination was tested on a 2 % Ultra Pure agarose (GIBCO™) or (SEA KEM LE™) pre-stained with ethidium bromide (0.25μg/ml) or on a 6 % denaturing polyacrylamide gel respectively. The complete optimised protocols are detailed above.

PREPARATION OF STOCK SOLUTIONS

Preparation of 40 % acrylamide stock solution

The 40% acrylamide (19:1) stock solution used, was prepared by mixing 38gr of acrylamide (CH_2: $CH.NH_2$) with 2 gr Bis-Acrylamide (CH2: CH.CO.NH) $_2CH_2$). The mixture was dissolved in a total volume of 100ml solution (pH 7.0) with the addition of distilled deionised H_2O. The mixture was deionised over night with 0.2 volume of monobed resin, followed by filtration through Whatman No.1 paper. Deionisation was necessary as acrylamide (CH_2: $CH.NH_2$) and Bis-Acrylamide (CH2: CH.CO.NH) $_2CH_2$) are often contaminated with metal ions during the manufacturing process.

Preparation of 5×TBE stock solution per litre

5 × TBE stock solution was prepared by mixing 54 g Tris base with 27.5 g boric acid (H_3BO_3) and 20 ml EDTA (0.5 M) pH 8.0. The mixture was dissolved in a total volume of 1 litre with distilled deionised H_2O.

Preparation of 10% Ammonium Persulphate Stock solution

10% Ammonium Persulfate was prepared by dissolving 1gr ammonium persulphate $(NH_4)_2S_2O_8$ in a total volume of 10ml H_2O. The stock solution was dispensed in 1.5 ml vials and stored in –20 °C.

Preparation of EDTA (0.5 M) solution

EDTA (0.5 M) prepared by dissolving 18.6 g EDTA and 21 pellets of sodium hydroxide in 100ml of distilled deionised H_2O. The solution was adjusted to pH 8.0

RESULTS

RESULTS

DQβ1 results methodology

The protocol employed for this type of experiment was a standard *All*Set™ SSP "Low resolution" tissue typing procedure for DQβ1typing, purchased as a kit from Dynal Ltd, UK. The allelic specificity for each serotype is presented in Table 2. Based on sequence similarities, the allelotype DQβ*0305 and DQβ*0307 will type serologically as DQ 8 (DQβ*0302) and that DQβ*0306 will type serologically as DQ 9 (DQβ*03032).

The serotype DQ 7 incorporating the allelotypes DQβ*0301, DQβ*0304, the serotype DQ 8 incorporating the allelotype DQβ*0302 and the serotype DQ 9 incorporating the allelotype DQβ*03032 will type serologically as DQ 3 that is incorporate the allelotypes DQβ*0301 to DQβ*0307 (Table 2). Therefore, any probable DQ 7, DQ 8, DQ 9 predicted serotype results will be accounted as serotype DQ 3, the reason being that it is impossible to identify specific allelotypes for these three former serotypes utilising an *All*Set™ SSP "Low resolution" DQβ1 typing kit. Additionally, all the serotypes incorporate a group of alleles (see Table 2). Thus, it is impossible to determine precisely specific DQβ1allelotypes. Therefore, all the DQβ1data obtained will be presented as a serotype with correspondence to the allelotypes in Table 2. If such specificity was to be determined specifically an *All*Set™ SSP "High resolution" DQβ1typing protocol must have been employed but it is very expensive to do so.

The DQβ1 locus

The results at this locus are presented in Table 3. Among the entire test populations and the control population the serotype DQ 2 * 3 in benign Barrett's (32.4 %) presents the highest expresion in frequency. The results show that the serotype DQ 2 * 3 is by far the most commonly expressed in control population (20.7%) and also in the three test

populations gastric cancer (22.7%), benign Barrett's (32.4%) and malignant Barrett's (19.6%). However, no significant association is present. Within the control population the serotypes DQ 2 * 6 (13.9%) and DQ 3 * 6 (13.9%) are the second most frequently expressed. In benign Barrett's population the serotype DQ 2 * 6 is the second most frequently expressed serotype (16.2%) where in the other two test populations gastric cancer and malignant Barrett's the expresion is lower (6.8%) and (9.8%) respectively. However, DQ 2 * 6 serotype in benign Barrett's population present higher expresion (16.2%) than it is in control population (13.9%). Although, significant association was not found. The serotype DQ 3 * 6 in the control population (13.9%) compared with the gastric cancer population (13.6%) presents very similar expresion. The other two-test populations for the former serotype show much lower expresion and no significance is present. The homozygous serotype DQ 3 * 3 in the gastric cancer population (15.9%) is the second most frequently expressed when compared with the control population and the other two test populations presents higher expresion. However the homozygous serotype DQ 3 * 3 is the second most frequently expressed serotype in malignant Barrett's population (13.7%) but if compared with the control, the gastric and the benign Barrett's population the difference is very low and no significance is present.

This expression pattern is also reflected in the allelotype expression pattern presented in Table 4. The allelotype expression at this locus shows that the serotype DQ 3 in the gastric cancer population (39.8%) to be the most commonly expressed among all the other serotypes and among all types of population tested. The DQ 3 serotype expresion in the benign Barrett's population and in the malignant Barrett's population presents lower frequencies of (33.8%) and (33.3%) compared with the gastric cancer and the control population. However, in the control population the serotype DQ 3 presents the lowest frequency of (28.5%) if compared with all three-test populations where significant association is present between the control and gastric cancer population (Ch^2 =4.133, P=0.0421). No other associations were found among the rest of the populations. The

frequency of DQ 2 serotype in the benign Barrett's population (36.5%) is the second most frequently expressed serotype among the all the test populations. With regards to DQ 2 serotype expresion in the malignant Barrett's population (32.3%) and in gastric cancer population (28.4%), the results show that malignant Barrett's population presents slightly higher expresion. Comparing the serotype DQ 2 in the control population (30.5%) with the former two test populations, the malignant Barrett's population (32.3%) presents a slightly higher expresion than the control population where the gastric cancer population (28.4%) presents a slightly lower expresion than the control population for the serotype DQ 2. However, significant associations were not found. Additionally the serotype DQ 6 in the control population (26.0 %) presents higher expresion than in the three test populations, gastric cancer population (20.4%), benign Barrett's population (16.2%) and malignant Barrett's population (16.7%). Among the three test populations, gastric cancer population has the highest DQ 6 serotype expresion (20.4%) whereas benign Barrett's population and malignant Barrett's population if compared with each other, present no significant difference or association. Representative gel pictures of HLA-DQβ1 genotyping by DQ "low resolution" PCR-SSP (Dynal AllSet ™) are illustrated in Fig 10.

TABLE 3. Distribution and relative frequency (%) of DQβ1 genotypes in normal individuals and three test populations.

Predicted DQβ1 serotype**	Distribution and Relative frequency (%)			
	Control* n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
2 * 2	8 (7.9)	4 (9.1)	3 (8.1)	6 (11.8)
2 * 3	21 (20.7)	10 (22.7)	12 (32.4)	10 (19.6)
2 * 4	2 (2.0)	1 (2.3)	0 (0.0)	0 (0.0)
2 * 5	8 (7.9)	3 (6.8)	3 (8.1)	6 (11.8)
2 * 6	14 (13.9)	3 (6.8)	6 (16.2)	5 (9.8)
3 * 3	9 (8.9)	7 (15.9)	3 (8.1)	7 (13.7)
3 * 4	1 (1.0)	0 (0.0)	1 (2.7)	1 (2.0)
3 * 5	3 (3.0)	5 (11.4)	4 (10.8)	6 (11.8)
3 * 6	14 (13.9)	6 (13.6)	2 (5.4)	3 (5.9)
4 * 5	2 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)
4 * 6	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
5 * 5	3 (3.0)	0 (0.0)	0 (0.0)	1 (2.0)
5 * 6	7 (6.9)	0 (0.0)	2 (5.4)	3 (5.8)
6 * 6	9 (8.9)	4 (9.1)	1 (2.7)	3 (5.8)

n= number of subjects

* Obtained from the west of Scotland tissue typing laboratory, Glasgow Royal Infirmary.

**The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 4. Total distribution and relative frequency (%) of DQβ1 genotypes in normal individuals and three test populations.

Predicted DQβ1 serotype**	Total genotypic distribution and relative frequency (%)			
	Control* n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
2	61 (30.5)	25 (28.4)	27 (36.5)	33 (32.3)
3	56 (28.5)♣	35 (39.8)♣	25 (33.8)	34 (33.3)
4	5 (2.0)	2 (2.3)	1 (1.3)	1 (1.0)
5	26 (13.0)	8 (9.1)	9 (12.2)	17 (16.7)
6	53 (26.0)	18 (20.4)	12 (16.2)	17 (16.7)

n= number of alleles

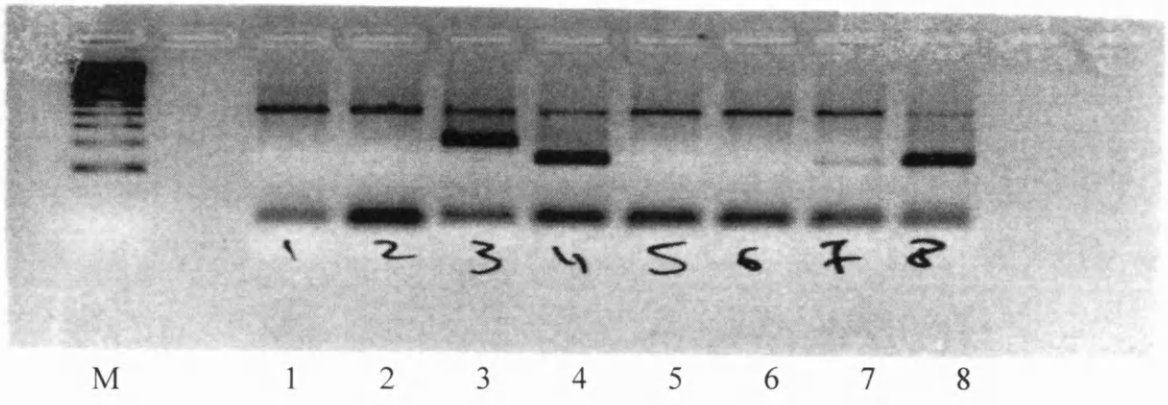
* Obtained from the west of Scotland tissue typing laboratory, Glasgow Royal Infirmary.

**The DQβ1 genotype and serological relationship is presented in Table 2.

♣ Significant difference between test group and controls ($P < 0.05$)

($Ch^2 = 4.133$, $P=0.0421$)

Panel A



Panel B

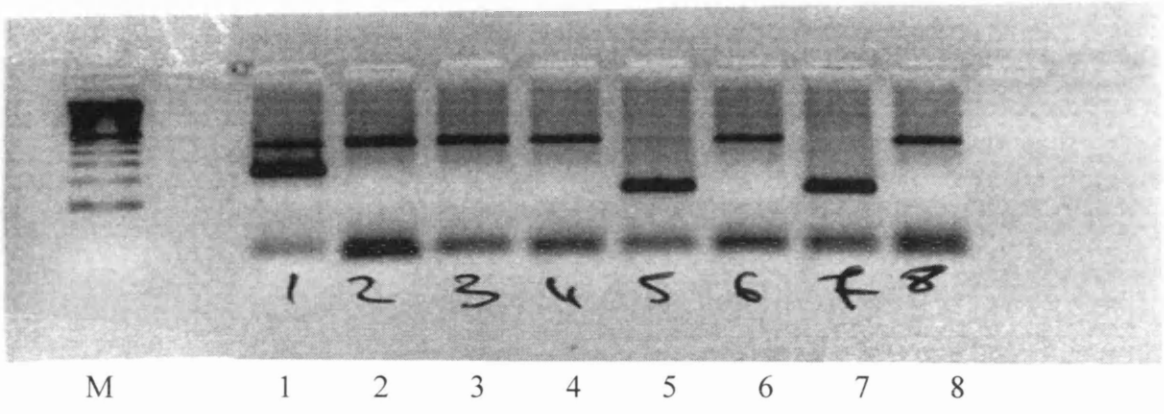


Fig 10. Illustration of typical HLA-DQB1 genotyping by DQ "low resolution" PCR-SSP (Dynal AllSet ™). The direction of electrophoretic migration is downward. The internal positive control primer pair amplifies a 429bp segment of the human growth hormone gene in all lanes 1-8. In the presence of a specific amplification, the intensity of the control band often decreases. Alleles are assigned by the presence of PCR product. The PCR product sizes (see table 2) may also be helpful in the interpretation of DQB1 typing. Lane M indicates molecular weight marker in base pair (100bp).

Panel A: Positive lanes: 3, 4, and 8. Genotype: DQB1*0201-*0203, DQB1*0201, DQB1*0401, *0402. Serotype: DQ 2, DQ 4.

Panel B: Positive lanes: 1, 5, and 7. Genotype: DQB1*0501-*0504, DQB1*0301, *0304, DQB1*0301. Serotype: DQ 5, DQ 7.

The HSP 70-2 locus

The results at this locus are presented in Table 5. Among the entire test populations and the control population the heterozygous genotype 1 * 2 in gastric cancer population (61.9 %) presents the highest expresion. However, the results show that the heterozygous genotype 1 * 2 is not the only most commonly expressed, in gastric cancer population (61.9%) but also in the other two test populations benign Barrett's (57.7%), malignant Barrett's (30.0%) and in the control population (54.1%). The malignant Barrett's population (30.0%) presents much lower frequency than the control population (54.1%), where significant association was found ($Ch^2 = 5.468$, P= 0.0194) between these two. Comparing the benign Barrett's and gastric cancer populations with the control population for the heterozygous genotype 1 * 2 their frequencies are slightly higher. Additionally the malignant Barrett's population (30.0%) presents much lower expresion compared with the gastric cancer population (61.4%) with difference of (31.9%) but significant association was not found.

The homozygous genotype 2 * 2 is the second most expressed genotype among all the other genotypes. The homozygous genotype 2 * 2 in malignant Barrett's population (43.3%) presents the highest expresion compared with the two other test populations, benign Barrett's (26.9%), gastric cancer (33.3%) and in the control population (34.2%). The difference between the benign Barrett's (26.9%) and the gastric cancer (33.3%) is not significant. Similarly the difference of those two former test populations compared with the control population is not significant regarding the homozygous genotype 2 * 2. The difference in frequency between the benign Barrett's (26.9%) and the malignant Barrett's population (43.3%) is also not significant.

This expression pattern is also reflected in the allelotype expression pattern presented in Table 6. The allelotype expression at this locus shows that the allelotype 2* = 936bp is the most commonly expressed in all types of population tested. Specifically the allelotype 2* = 936bp in gastric cancer population (64.3%) presents the highest expresion

compared with the other two test populations benign Barrett's (55.8%), malignant Barrett's (58.34%) and the control population (61.3%) but significant associations were not found. However, the allelotype 2* = 936bp presents higher expresion in the control population (61.3%) compared with the two test populations benign Barrett's (55.8%), malignant Barrett's (58.34%) but significant association was not found. Additionally the difference among the benign Barrett's (55.8%), malignant Barrett's (58.34%) and gastric cancer population (64.3%) regarding the allelotype 2* = 936bp is not great and significant associations were not found. A representative gel picture of amplified fragment length polymorphism of heat shock protein 70-2 (HSP70-2) gene is illustrated in Fig 11.

TABLE 5. Distribution and relative frequency (%) of HSP 70-2 genotypes in normal individuals and three test populations.

HSP 70-2 genotypes	Distribution and Relative frequency (%)			
	Control n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
1 * 1	13 (11.7)	2 (4.8)	4 (15.4)	8 (26.7)
1 * 2	60 (54.1)♣	26 (61.9)	16 (57.7)	9 (30.0)♣
2 * 2	38 (34.2)	14 (33.3)	7 (26.9)	13 (43.3)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n= number of subjects

♣ Significant difference between test group and controls ($P < 0.05$)

($Ch^2 = 5.468$, $P= 0.0194$)

TABLE 6. Total distribution and relative frequency (%) of HSP 70-2 allelotypes in normal individuals and three test populations.

HSP 70-2 allelotypes	Total allelotypic distribution and relative frequency (%)			
	Control n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
1*	86 (38.7)	30 (35.7)	23 (44.2)	25 (41.66)
2*	136 (61.3)	54 (64.3)	29 (55.8)	35 (58.34)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n= number of alleles

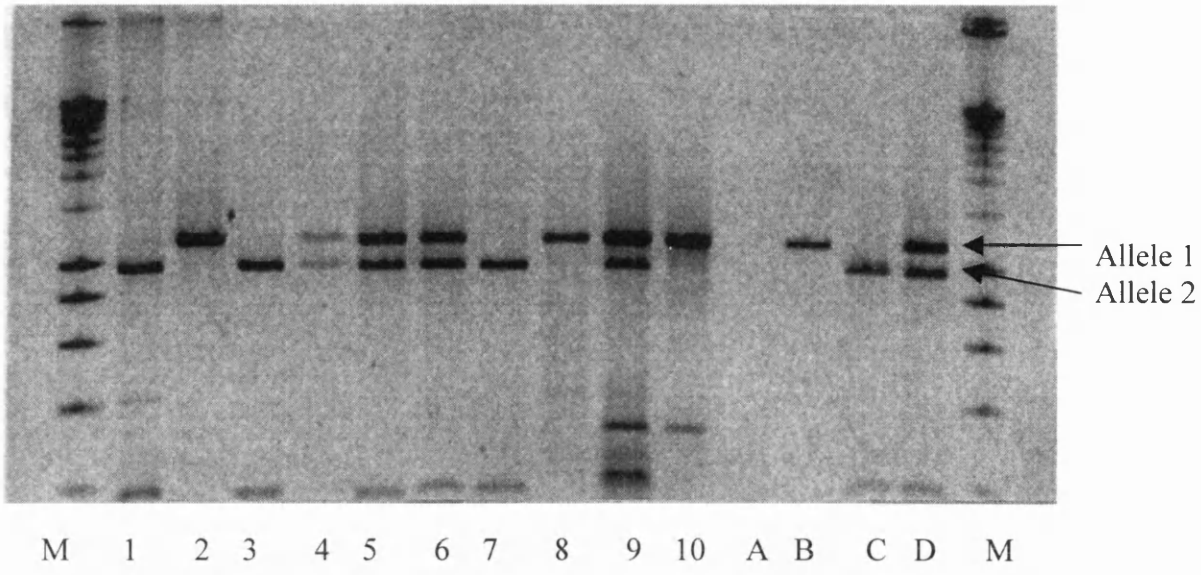


Fig 11. Amplified fragment length polymorphism of heat shock protein 70-2 (HSP70-2) gene. The (HSP70-2) polymerase chain reaction (PCR) products were digested with restriction digest enzyme PST-1 (5′- CTGCA -3′ and (5′- GACGTC -3′) and separated on a 2% ultra pure agarose gel. The HSP allele 1 corresponds to the 1117 bp and the allele 2 to 936 bp. The identification analysis of RFLP repeats of all the samples performed in comparison with representative control fragments (cell line DNA); Lanes, A (H_2O), B (homozygote-1117 bp), C (homozygote-936 bp), D (heterozygote-1117 bp – 936 bp), and against molecular marker M (ϕx174 -RF DNA/ Hαe III).

The D6S273 locus

The results at this locus are presented in Table 7. Among the entire test populations and the control population the heterozygous microsatellite genotype 132-134 in malignant Barrett's population (28.1 %) presents the highest expression of all. Additionally the results show that the heterozygous microsatellite genotype 132-134 is by far the most commonly expressed in malignant Barrett's population (28.1%) and also in the gastric cancer population (24.3%) and in the control population (12.4%) among the rest of genotypes. In the benign Barrett's population the frequency of the genotypic expresion is widely distributed among the heterozygous microsatellite genotypes 126-134, 130-132, 130-134, 132-138, all with equal frequencies of (15.3%). The heterozygous microsatellite genotype 132-134 presents a small difference in frequency among the malignant Barrett's population (28.1%) and the gastric cancer population (24.3%). However, the difference in frequency between the gastric cancer population (24.3%) and the control is high (11.9%) but a significant association was not found. The difference in frequency for the heterozygous microsatellite genotype 132-134, among the control population (12.4%) and the malignant Barrett's population (28.1%) however is higher (15.7%) where significant association was found (Ch^2 =4.510, P=0.0337).

This expression pattern is also reflected in the microsatellite allelotype expression pattern presented in Table 8. The microsatellite allelotype expression at this locus shows that the microsatellite allelotype 134 is the most commonly expressed among all the other allelotypes in all populations tested. The microsatellite allelotype 134 in gastric cancer population (37.9%) presents slightly higher expresion than the other two test populations benign Barrett's (34.6%), malignant Barrett's (32.9%) and the control population (26.7%). However, the microsatellite allelotype 134 presents no significant differences in frequency and no significant association is present between the benign Barrett's population (34.6%) and malignant Barrett's (32.9%). However, these two-test populations present a slightly

higher expresion if compared with the control population (26.7%) but significant association was not found.

In the control population the microsatellite allelotype 132 presents slightly higher frequency (28.1%) compared with the microsatellite allelotype 134 (26.7%). The microsatellite allelotype 132 in malignant Barrett's population (29.7%) presents higher expresion compared with the benign Barrett's population (19.2%), the gastric cancer population (18.9%) and but not if compared with the control population (28.1%) but significant associations were not found. Additionally, the microsatellite allelotype 132 presents no significant differences in frequency between the benign Barrett's population (19.2%) and the gastric cancer population (18.9%) but these two-test populations present lower expresion if compared with the control population (28.1%). However significant associations were not found among these. A representative autoradiograph of microsatellite typing at tested 6p (D6S273) locus is illustrated in Fig 12.

TABLE 7. Distribution and relative frequency (%) of D6S273 genotypes in normal individuals and three test populations.

D6S273 genotypes	Distribution and Relative frequency (%)			
	Control n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
126 – 126	2 (1.9)	1(2.7)	0 (0.0)	1 (3.1)
126 – 128	1(0.9)	0 (0.0)	0 (0.0)	0 (0.0)
126 – 130	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)
126 – 132	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)
126 – 134	2 (1.9)	2 (5.4)	2 (15.3)	1 (3.1)
126 – 136	1(0.9)	0 (0.0)	0 (0.0)	0 (0.0)
126 – 138	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)
128 – 128	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)
128 – 130	5 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)
128 – 132	3 (2.9)	1 (2.7)	0 (0.0)	1 (3.1)
128 – 134	0 (0.0)	3 (8.1)	0 (0.0)	1 (3.1)
128 – 136	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)
128 – 138	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)
130 – 130	3 (2.9)	0 (0.0)	0 (0.0)	1 (3.1)
130 – 132	3 (2.9)	1 (2.7)	2 (15.3)	2 (6.2)
130 – 134	6 (5.7)	3 (8.1)	2 (15.3)	3 (9.4)
130 – 136	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)

132 – 132	12 (11.4)	0 (0.0)	0 (0.0)	2 (6.2)
132 – 134	13 (12.4)♣	9 (24.3)	0 (0.0)	9 (28.1)♣
132 – 136	4 (3.8)	0 (0.0)	1 (7.8)	0 (0.0)
132 – 138	9 (8.6)	3 (8.1)	2 (15.3)	3 (9.4)
134 – 134	10 (9.5)	4 (10.8)	1 (7.8)	3 (9.4)
134 – 136	5 (4.8)	1 (2.7)	1 (7.8)	1 (3.1)
134 – 138	10 (9.5)	2 (5.4)	2 (15.3)	0 (0.0)
136 – 136	2 (1.9)	2 (5.4)	0 (0.0)	0 (0.0)
136 – 138	2 (1.9)	2 (5.4)	0 (0.0)	1 (3.1)
138 – 138	0 (0.0)	2 (5.4)	0 (0.0)	1 (3.1)

n = number of subjects

♣ Significant difference between test group and controls ($P < 0.05$)

($Ch^2 = 4.510$, $P=0.0337$)

TABLE 8. Total distribution and relative frequency (%) of D6S273 allelotypes in normal individuals and three test populations.

D6S273 allelotypes	Total allelotypic distribution and relative frequency (%)			
	Control n (%)	Gastric cancer n (%)	Benign Barrett's n (%)	Malignant Barrett's n (%)
126	14 (6.7)	4 (5.4)	2 (7.7)	4 (6.2)
128	15 (7.1)	5 (6.8)	0 (0.0)	3 (4.7)
130	26 (12.4)	4 (5.4)	4 (15.4)	7 (10.9)
132	59 (28.1)	14 (18.9)	5 (19.2)	19 (29.7)
134	56 (26.7)	28 (37.9)	9 (34.6)	21 (32.9)
136	19 (9.0)	7 (9.4)	2 (7.7)	3 (4.7)
138	21 (10.0)	12 (16.2)	4 (15.4)	7 (10.9)

n = number of alleles

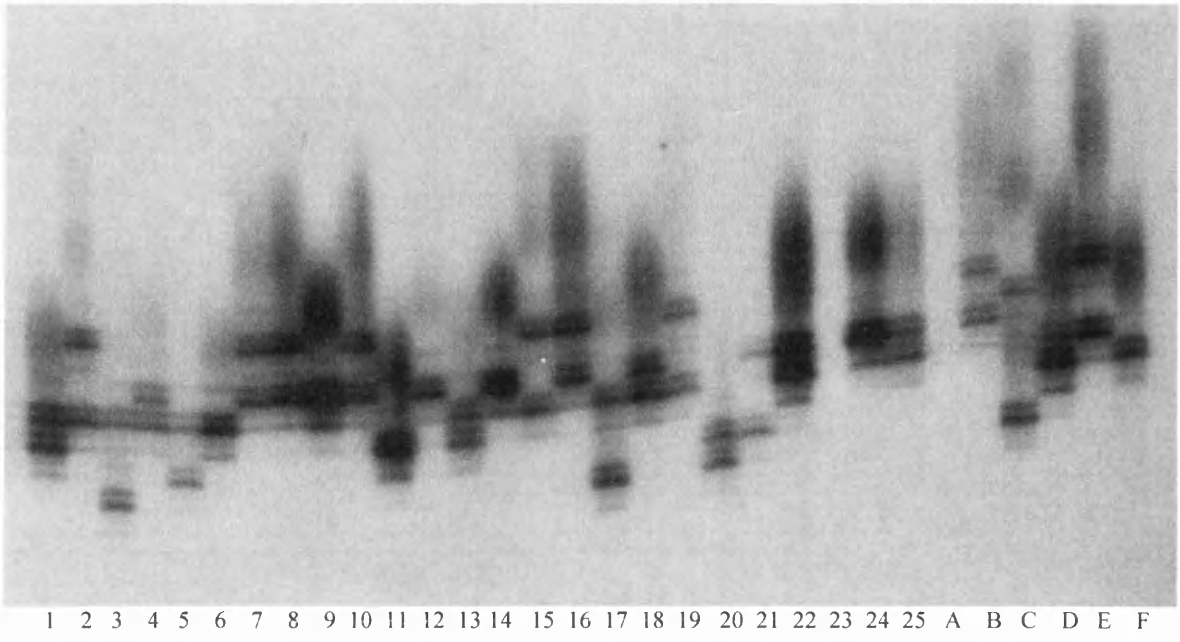


Fig 12. Representative autoradiograph of microsatellite typing at tested 6p (D6S273) locus. The identification analysis of microsatellite repeats of all the samples performed in comparison with representative control fragments (cell line DNA); Lanes, A (H_2O), B (heterozygote-134 -138), C (heterozygote-126 -136), D (heterozygote-128 -130), E (heterozygote-132 -138), F (homozygote-130).

DQβ1 predicted serotypes in comparison with TNFa 1-14 microsatellites

It would be interesting to examine the relationship between various TNFa 1-14 microsatellite alleles with DQβ1predicted serotypes (genotypes are given in Table 2), looking for linkage between them in the three test populations benign Barrett's, malignant Barrett's and gastric cancer population. (I would like to thank Hasan Kasem for the provision of the TNFa data on which I did this analysis).

Since these two polymorphic sites lie within a close distance of each other, telomeric of the short arm on Chromosome 6 in region 2, band 1, sub-band 3, it was deemed reasonable to examine whether, in any of the three test populations, patterns pertaining to disease specificity could be observed and to investigate for linkage between DQβ1predicted serotypes and TNFa microsatellite allelotypes at the three test sites.

The results are presented in Tables 9 to 11. The data obtained in those Tables 9 to 11, generally indicates that there is a wide distribution of DQβ1predicted serotypes and microsatellite allelotypes. However the results in Table 9 present a higher frequency for DQ 2 – TNFa2 in benign Barrett's population (15.7%) compared with the rest of the DQβ1predicted serotypes and TNFa microsatellite alleles in this population. Similarly, the results in Table 10 present a higher frequency for DQ 2 – TNFa2 in malignant Barrett's population (16.8%) compared with the rest of the DQβ1predicted serotypes and TNFa microsatellite alleles in this population. Again the results in Table 11 present a higher frequency for DQ 2 – TNFa2 in gastric cancer population (11.6%) compared with the rest of the DQβ1predicted serotypes and TNFa microsatellite alleles in this population.

Comparing the DQ 2 – TNFa2 results among the benign Barrett's population (15.7%) and malignant Barrett's population (16.8%) not significant difference in frequencies is present and significant association was not found. However, the DQ 2 – TNFa2 results for the benign Barrett's population (15.7%) and the malignant Barrett's population (16.8%) if compared with the gastric cancer population (11.6%), present higher

frequencies with differences of (4.1%) and (5.2%) respectively but again significant association was not found. These two differences are not major among the last three former test populations that were tested.

TABLE 9. Distribution and relative freq uency (%) of DQβ1 alleles in comparison with TNFa 1-14 microsatellites in benign Barrett's oesophagus.

DQβ1 predicted serotype	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**$TNFa_8$**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**$TNFa_{14}$**
2*	0 (0.0)	11 (15.7)	0 (0.0)	2 (2.9)	3 (4.3)	3 (4.3)	5 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	1(1.4)	0 (0.0)	0 (0.0)	0 (0.0)
3*	0 (0.0)	3 (4.3)	0 (0.0)	2 (2.9)	0 (0.0)	5 (7.1)	1 (1.4)	1 (1.4)	0 (0.0)	4 (5.7)	6 (8.6)	0 (0.0)	2 (2.9)	0 (0.0)
4*	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5*	0 (0.0)	4 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.9)	1 (1.4)	0 (0.0)	0 (0.0)	1 (1.4)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)
6*	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.9)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	1 (1.4)	1 (1.4)	6 (8.6)	0 (0.0)	0 (0.0)	0 (0.0)

n = 70 alleles

*The DQβ1 genotype and serological rel ationship is presented in Table 2.

TABLE 10. Distribution and relative frequency (%) of DQβ1 genotypes in comparison with TNFa 1-14 microsatellites in malignant Barrett's oesophagus.

DQB1 predicted serotypes	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**$TNFa_8$**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**$TNFa_{14}$**
2*	0 (0.0)	17 (16.8)	0 (0.0)	5 (4.9)	1 (0.99)	4 (3.9)	2 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.9)	0 (0.0)	0 (0.0)	0 (0.0)
3*	0 (0.0)	9 (8.9)	0 (0.0)	2 (1.9)	4 (4.0)	5 (4.9)	2 (1.9)	0 (0.0)	0 (0.0)	3 (3.0)	6 (5.9)	0 (0.0)	3 (3.0)	0 (0.0)
4*	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.99)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5*	1 (0.99)	9 (8.9)	0 (0.0)	1 (0.99)	1 (0.99)	1 (0.99)	1 (0.99)	0 (0.0)	0 (0.0)	1 (0.99)	1(0.99)	0 (0.0)	1 (0.99)	0 (0.0)
6*	0 (0.0)	4 (3.9)	0 (0.0)	1 (0.99)	0 (0.0)	1 (0.99)	2 (1.9)	1 (0.99)	0 (0.0)	0 (0.0)	6 (5.9)	1 (0.99)	1 (0.99)	0 (0.0)

n = 101 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 11. Distribution and relative frequency (%) of DQβ1 genotypes in comparison with TNFa 1-14 microsatellites in gastric cancer population.

DQB1 Predicted serotypes	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**$TNFa_8$**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**$TNFa_{14}$**
2*	0 (0.0)	10 (11.6)	2 (2.3)	2 (2.3)	1 (1.2)	2 (2.3)	3 (3.5)	1 (1.2)	0 (0.0)	1 (1.2)	2 (2.3)	0 (0.0)	1 (1.2)	0 (0.0)
3*	1 (1.2)	6 (7.0)	0 (0.0)	1 (1.2)	5 (5.8)	6 (7.0)	3 (3.5)	1 (1.2)	3 (3.5)	2 (2.3)	6 (7.0)	1 (1.2)	0 (0.0)	0 (0.0)
4*	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
5*	0 (0.0)	3 (3.5)	0 (0.0)	1 (1.2)	1 (1.2)	2 (2.3)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
6*	0 (0.0)	5 (5.8)	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.3)	7 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)

n = 86 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

HSP 70-2 allelotypes in comparison with TNFa 1-14 microsatellites

Additionally, it would be interesting to examine the relationship between various TNFa 1-14 microsatellite alleles with HSP 70-2 allelotypes, looking for linkage between them in the three test populations benign Barrett's, malignant Barrett's and gastric cancer population.

Since these two polymorphic sites lie within close distance of each other telomeric on the short arm of Chromosome 6 in region 2, band 1, sub-band 3, it was deemed reasonable to examine whether, in any of the three test populations, patterns pertaining to disease specificity could be observed and to investigate for linkage between HSP 70-2 allelotypes and TNFa microsatellite allelotypes at the three test sites.

The results are presented in Tables 12 to 14. The results in Table 12 present a higher frequency for allele 1*(1117bp) – TNFa2 in benign Barrett's population (23.1%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population. However the allele 2*(936 bp) – TNFa11 also presents an increased frequency of (15.4%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population but lower than allele 1*(1117bp) – TNFa2. Similarly, the results in Table 13 present a higher frequency for allele 1*(1117bp) – TNFa2 in malignant Barrett's population (21.7%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population. However the allele 2*(936 bp) – TNFa2 presents an increased frequency of (20.0%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population but slightly lower than allele 1*(1117bp) – TNFa2. The results in Table 14 present a higher frequency for allele 1*(1117bp) – TNFa2 in gastric cancer population (20.0%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population. However the allele 2*(936 bp) – TNFa11 presents an increased frequency of (20.2%) compared with the rest of the HSP 70-2 alleles and TNFa microsatellite alleles in this population and with equal frequency with allele 1*(1117bp) – TNFa2.

Comparing the allele 1*(1117bp) – TNFa2 results among the benign Barrett's population (23.1%), malignant Barrett's population (21.7%) and gastric cancer population (20.2%), no major difference in frequencies are present and significant association was not found. Again comparing the allele 2*(936bp) – TNFa11 results among the benign Barrett's population (15.4%) and gastric cancer population (20.2%) a small difference of (4.8%) in frequencies are present but significant association was not found. Additionally the genotype, allele 2*(936bp) – TNFa2 presents the highest frequency in malignant Barrett's population (20.0%) compared with the benign Barrett's population (9.6%) and with the gastric cancer population (14.2%) but again significant associations were not found. Additionally, there is a small difference in frequency among the benign Barrett's population (9.6%) and the gastric cancer population (14.2%) but significant association was not found.

TABLE 12. Distribution and relative frequency (%) of HSP 70-2 alleles in comparison with TNFa 1-14 microsatellites in benign Barrett's oesophagus.

HSP 70-2 alleles	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**T NFa_8**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**TNFa $_{14}$**
1*	0 (0.0)	12 (23.1)	0 (0.0)	4 (7.7)	1 (1.9)	1 (1.9)	3 (5.8)	0 (0.0)	0 (0.0)	1 (1.9)	1(1.9)	0 (0.0)	0 (0.0)	0 (0.0)
2*	0 (0.0)	5 (9.6)	0 (0.0)	2 (3.8)	0 (0.0)	7 (13.5)	2 (3.8)	1 (1.9)	0 (0.0)	3 (5.8)	8 (15.4)	0 (0.0)	1 (1.9)	0 (0.0)

Allele 1* = 11 17 bp

Allele 2* = 93 6 bp

n = 52 alleles

TABLE 13. Distribution and relative frequency (%) of HSP 70-2 alleles in comparison with TNFa 1-14 microsatellites in malignant Barrett's oesophagus.

HSP 70-2 alleles	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**$TNFa_8$**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**$TNFa_{14}$**
1*	0 (0.0)	13 (21.7)	0 (0.0)	5 (8.3)	2 (3.3)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
2*	0 (0.0)	12 (20.0)	0 (0.0)	3 (5.0)	2 (3.3)	4 (6.7)	4 (6.7)	0 (0.0)	0 (0.0)	4 (6.7)	5 (8.3)	1 (1.7)	3 (5.0)	0 (0.0)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 60 alleles

TABLE 14. Distribution and relative frequency (%) of HSP70-2 alleles in comparison with TNFa 1-14 microsatellites in gastric cancer population.

HSP 70-2 alleles	**$TNFa_1$**	**$TNFa_2$**	**$TNFa_3$**	**$TNFa_4$**	**$TNFa_5$**	**$TNFa_6$**	**$TNFa_7$**	**$TNFa_8$**	**$TNFa_9$**	**$TNFa_{10}$**	**$TNFa_{11}$**	**$TNFa_{12}$**	**$TNFa_{13}$**	**$TNFa_{14}$**
1*	1 (1.2)	17 (20.2)	1 (1.2)	3 (3.6)	3 (3.6)	2 (2.4)	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
2*	0 (0.0)	12 (14.3)	0 (0.0)	4 (4.8)	0 (0.0)	9 (10.7)	3 (3.6)	1 (1.2)	1 (1.2)	5 (5.9)	17 (20.2)	1 (1.2)	0 (0.0)	0 (0.0)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 84 alleles

HSP 70-2 allelotypes in comparison with DQβ1 predicted serotypes

Also it would be interesting to examine the relationship between various DQβ1 predicted serotypes with HSP 70-2 allelotypes, looking for linkage between them in the three test populations benign Barrett's, malignant Barrett's and gastric cancer population.

Since these two polymorphic sites lie within close proximity, among them telomeric on the short arm of Chromosome 6 in region 2, band 1, sub-band 3, it was deemed reasonable to examine whether, in any of the three test populations, patterns pertaining to disease specificity could be observed and to investigate for linkage between HSP 70-2 allelotypes and various DQβ1 predicted serotypes at the three test sites.

The results are presented in Tables 15 to 17. The results in Table 15 present a higher frequency for allele 1*(1117bp) – DQ2 in benign Barrett's population (28.8%) compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes in this population. However the allele 2*(936 bp) – DQ3 also presents an increased frequency of (25.0%) compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes in this population but lower than allele 1*(1117bp) – DQ2. Similarly, the results in Table 16 present an increased frequency for allele 1*(1117bp) – DQ2 in malignant Barrett's population (15.0%) compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes where surprisingly the allele 2*(936 bp) – DQ2 presents an equal frequency of (15.0%) in this population. However, the allele 2*(936 bp) -DQ3 presents the most increased frequency of (30.0%) compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes in malignant Barrett's population, double the frequency of allele 1*(1117bp) – DQ2 and 2*(936 bp) – DQ2. The results in Table 17 present the highest frequency for the allele 2*(936 bp) – DQ3 in gastric cancer population (30.6%) compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes in this population. However, the allele 1*(1117bp) – DQ2 presents an increased frequency of (22.2%)

compared with the rest of the HSP 70-2 alleles and DQβ1 predicted serotypes in this population.

Comparing the allele 2*(936 bp) - DQ3 results between the malignant Barrett's population (30.0%) and gastric cancer population (30.6%), no major difference in frequencies is present and significant association was not found. Again comparing the allele 2*(936bp) – DQ3 results among the benign Barrett's population (25.0%), malignant Barrett's population (30.0%) and gastric cancer population (30.6%) there is a small difference of (5.0%) and (5.6%) in frequencies present and significant association was not found. The allele 1*(1117bp) – DQ2 if compared among the three test populations presents the highest frequency in the benign Barrett's population (28.8%) where the lowest frequency is present in malignant Barrett's population (15.0%), less than half the benign Barrett's frequency but significant association was not found. The frequency of allele 1*(1117bp) – DQ2 in gastric cancer populations (22.2%) compared with the benign Barrett's population (28.8) and malignant Barrett's population (15.0%) presents a small difference and significant association was not found. However, a big difference in frequencies is present among the 2*(936bp) – DQ2 malignant Barrett's population (15.0%) and 2*(936bp) – DQ2 gastric cancer population (2.7%) where significant association was found between them ($Ch^2 = 3.884$, $P= 0.0487$).

TABLE 15. Distribution and relative frequency (%) of HSP 70-2 alleles in comparison with DQβ1allels in benign Barrett's oesophagus.

HSP 70-2 alleles	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
1*	15 (28.8)	4 (7.7)	0 (0.0)	2 (3.8)	1 (1.9)
2*	6 (11.5)	13 (25.0)	0 (0.0)	5 (9.6)	6 (11.5)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 52 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 16. Distribution and relative frequency (%) of HSP 70-2 alleles in comparison with DQβ1alleles in malignant Barrett's oesophagus.

HSP 70-2 alleles	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
1*	9 (15.0)	6 (10.0)	0 (0.0)	5 (8.3)	3 (5.0)
2*	9 (15.0)♦	18 (30.0)	0 (0.0)	4 (6.7)	6 (10.0)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 60 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

♦Significant difference between two test populations, malignant Barrett's and gastric cancer ($P < 0.05$)

($Ch^2 = 3.884$, $P= 0.0487$)

TABLE 17. Distribution and relative frequency (%) of HSP 70-2 alleles in comparison with DQβ1alleles in gastric cancer population.

HSP 70-2 alleles	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
1*	8 (22.2)	2 (5.6)	0 (0.0)	1 (2.7)	3 (8.3)
2*	1 (2.7)♦	11 (30.6)	2 (5.6)	2 (5.6)	6 (16.7)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 36 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

♦Significant difference between two test populations, malignant Barrett's and gastric cancer ($P < 0.05$)

($Ch^2 = 3.884$, $P = 0.0487$)

DQβ1 predicted serotypes in comparison with D6S273 microsatellites

Again it would be interesting to examine the relationship between various D6S273 microsatellite alleles with DQβ1predicted serotypes (genotypes are given in Table 2), looking for linkage between them in the three test populations benign Barrett's, malignant Barrett's and gastric cancer population.

Since these two polymorphic sites lie within a very close distance among them telomeric on the short arm of Chromosome 6 in region 2, band 1, sub-band 3, it was deemed reasonable to examine whether, in any of the three test populations, patterns pertaining to disease specificity could be observed and to investigate for linkage between DQβ1predicted serotypes and D6S273 microsatellite allelotypes at the three test sites.

The results are presented in Tables 18 to 20. The results in Table 18 present a higher frequency for DQ 2 – 134 in benign Barrett's population (15.4%) compared with the rest of the DQβ1predicted serotypes and D6S273 microsatellite alleles in this population. The results in Table 19 present a higher frequency for DQ 3 – 134 in malignant Barrett's population (15.6%) compared with the rest of the DQβ1predicted serotypes and D6S273 microsatellite alleles in this population. Additionally, there is an increased frequency for DQ 2 – 132 in malignant Barrett's population (12.5%) something not seen in benign Barrett's population. The results in Table 20 present a higher frequency for DQ 3 – 134 in gastric cancer population (18.7%) compared with the rest of the DQβ1predicted serotypes and D6S273 microsatellite alleles in this population. Additionally there is an increased frequency for DQ 3 – 138 in gastric cancer population (12.5%) and equally for DQ 6 – 136 in gastric cancer population (12.5%), something not seen in the other two test populations.

Comparing the allele 132 - DQ2 results among the benign Barrett's population (7.7%), the malignant Barrett's population (12.5%) and gastric cancer population (3.1%), a major difference in frequencies is present between malignant Barrett's population (12.5%) and the gastric cancer population (3.1%) but significant association was not found. Now

comparing the allele 134 – DQ2 results among the benign Barrett's population (15.4%), the malignant Barrett's population (4.7%) and gastric cancer population (3.1%), a major difference in frequencies is present between benign Barrett's population (15.4%) and the gastric cancer population (3.1%) but significant association was not found. Comparing the allele 134 – DQ3 results among the benign Barrett's population (3.8%), the malignant Barrett's population (15.6%) and gastric cancer population (18.7%), again a major difference in frequencies is present between the benign Barrett's population (3.8%) and the gastric cancer population (18.7%) but significant association was not found.

Comparing the allele 138 – DQ3 results among the benign Barrett's population (3.8%), the malignant Barrett's population (4.7%) and gastric cancer population (12.5%), again a major difference in frequencies is present between the benign Barrett's population (3.8%) and the gastric cancer population (12.5%) but significant association was not found.

TABLE 18. Distribution and relative frequency (%) of D6S273 alleles in comparison with DQβ1predicted serotypes in benign Barrett's oesophagus.

D6S273 alleles	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
126	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)	1 (3.8)
128	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
130	1 (3.8)	0 (0.0)	0 (0.0)	3 (11.5)	0 (0.0)
132	2 (7.7)	3 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
134	4 (15.4)	3 (3.8)	0 (0.0)	0 (0.0)	3 (3.8)
136	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
138	0 (0.0)	3 (3.8)	0 (0.0)	0 (0.0)	1 (3.8)

n = 26 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 19. Distribution and relative frequency (%) of D6S273 alleles in comparison with DQβ1predicted serotypes in malignant Barrett's oesophagus.

D6S273 alleles	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
126	2 (3.1)	2 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
128	3 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
130	3 (4.7)	2 (3.1)	0 (0.0)	2 (3.1)	0 (0.0)
132	8 (12.5)	5 (7.8)	0 (0.0)	2 (3.1)	4 (6.2)
134	3 (4.7)	10 (15.6)	0 (0.0)	2 (3.1)	6 (9.4)
136	1 (1.6)	2 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
138	2 (3.1)	3 (4.7)	0 (0.0)	2 (3.1)	0 (0.0)

n = 64 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 20. Distribution and relative frequency (%) of D6S273 alleles in comparison with DQβ1predicted serotypes in gastric cancer population.

D6S273 genotypes	**DQ2***	**DQ3***	**DQ4***	**DQ5***	**DQ6***
126	2 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)
128	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
130	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
132	1 (3.1)	1 (3.1)	0 (0.0)	2 (6.2)	1 (3.1)
134	1 (3.1)	6 (18.7)	0 (0.0)	1 (3.1)	3 (9.4)
136	1 (3.1)	0 (0.0)	1 (3.1)	0 (0.0)	4 (12.5)
138	2 (6.2)	4 (12.5)	1 (3.1)	0 (0.0)	1 (3.1)

n = 32 alleles

*The DQβ1 genotype and serological relationship is presented in Table 2.

HSP 70-2 allelotypes in comparison with D6S273 microsatellites

The relationship between various D6S273 microsatellite alleles with HSP 70-2 allelotypes was examined looking for linkage in the three test populations benign Barrett's, malignant Barrett's, gastric cancer population and the control population.

Again these two polymorphic sites lie within a few base pairs (bp) distance of each other telomeric on the short arm of Chromosome 6 in region 2, band 1, sub-band 3, it seemed reasonable to examine whether, in any of the three test populations, patterns pertaining to disease specificity could be observed and to investigate for linkage between HSP 70-2 allelotypes and D6S273 microsatellite allelotypes at the three test sites.

The results are presented in Tables 21 to 24. The results in Table 21 show that the highest frequency occurs for allele 2*(936bp)-134 genotype in benign Barrett's population (27.3%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. However the allele 2*(936 bp)-130 genotype also presents an increased frequency of (9.1%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. The same frequency is present for the alleles 1*(1117bp)-130, 1*(1117bp)-132, 1*(1117bp)-138 genotypes indicating an equal distribution. The results in Table 22 show that the highest frequency occurs again for allele 2*(936bp)-134 genotype in the malignant Barrett's population (26.8%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. However, the allele 2*(936 bp)-130 genotype also presents an increased frequency of (17.9%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. The results in Table 23 show that the highest frequency occurs again for allele 2*(936bp)-134 genotype in the gastric cancer population (28.1%) compared with all the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. However, the allele 2*(936 bp)-130 also presents an increased frequency of (10.9%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. The results in Table 24 show that the highest frequency occurs for allele

2*(936bp)-132 genotype in control population (19.2%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population. The allele 2*(936 bp)-134 genotype also presents an increased frequency of (17.6%) compared with the rest of the HSP 70-2 alleles and D6S273 microsatellite alleles in this population.

Comparing the allele 2*(936 bp)-130 genotype results among the benign Barrett's population (9.1%), malignant Barrett's population (5.4%) the gastric cancer population (1.6%) and the controls (7.1%) the highest difference in frequencies is present between the benign Barrett's population and gastric cancer population but significant associations were not found. Comparing the allele 2*(936bp)-132 genotype results among the benign Barrett's population (4.5%), the malignant Barrett's population (17.9%), the gastric cancer population (10.9%)and the controls (19.2%), a major difference in frequency of (14.7%) is present between the controls (19.2%) and the benign Barrett's population (4.5%) but significant association was not found between these two and between the controls and the rest. Finally the frequencies for 2*(936bp)-134 among the benign Barrett's population (27.3%), malignant Barrett's population (26.8%) the gastric cancer population (28.1%) and the controls (17.6%) present no significant differences in frequency and significant associations were not found.

TABLE 21. Distribution and relative frequency (%) of HSP 70-2 allelotypes in comparison with D6S273 allelotypes in benign Barrett's oesophagus.

HSP 70-2 allelotypes	D6S273 allelotypes						
	126	128	130	132	134	136	138
1*	1 (4.5)	0 (0.0)	2 (9.1)	2 (9.1)	1 (4.5)	0 (0.0)	2 (9.1)
2*	0 (0.0)	0 (0.0)	2 (9.1)	3 (4.5)	6 (27.3)	1 (4.5)	1 (4.5)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 22 alleles

TABLE 22. Distribution and relative frequency (%) of HSP 70-2 allelotypes in comparison with D6S273 allelotypes in malignant Barrett's oesophagus.

HSP 70-2 allelotypes	D6S273 allelotypes						
	126	128	130	132	134	136	138
1*	4 (7.1)	1 (1.8)	3 (5.4)	6 (10.7)	4 (7.1)	1 (1.8)	3 (5.4)
2*	0 (0.0)	1 (1.8)	3 (5.4)	10 (17.9)	15 (26.8)	1 (1.8)	4 (7.1)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 56 alleles

TABLE 23. Distribution and relative frequency (%) of HSP 70-2 allelotypes in comparison with D6S273 allelotypes in gastric cancer population.

HSP 70-2 allelotypes	D6S273 allelotypes						
	126	128	130	132	134	136	138
1*	3 (4.7)	4 (6.2)	3 (4.7)	4 (6.2)	5 (7.8)	2 (3.1)	3 (4.7)
2*	1 (1.6)	1 (1.6)	1 (1.6)	7 (10.9)	18 (28.1)	3 (4.7)	9 (14.1)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 64 alleles

TABLE 24. Distribution and relative frequency (%) of HSP 70-2 allelotypes in comparison with D6S273 allelotypes in control population.

HSP 70-2 allelotypes	D6S273 allelotypes						
	126	128	130	132	134	136	138
1*	11 (6.4)	9 (4.9)	10 (5.5)	20 (11.0)	15 (8.2)	3 (1.6)	2 (1.1)
2*	2 (1.1)	4 (2.2)	13 (7.1)	35 (19.2)	32 (17.6)	13 (7.1)	13 (7.1)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n = 182 alleles

Comparison of the DQβ1 predicted serotypes in total Barrett's oesophagus patient population with the control population

The results at this locus are presented in Table 25. Among the entire Barrett's population the serotype DQ 2 * 6 (12.5 %) presents the highest expresion. The results show that the serotype DQ 2 * 3 is by far the most commonly expressed in control population (20.7%). Comparing the serotype DQ 2 * 6 (12.5 %) in Barrett's population with the serotype DQ 2 * 6 (13.9) in the control population the difference in frequency is very small and significant association was not found. However, comparing the serotype DQ 2 * 3 in control population (20.7%) with the serotype DQ 2 * 3 in Barrett's population (11.4%) the data reveals a difference in frequency of (9.3%) but significant association was not found. Similarly the data reveals a difference in frequency of (8.4%) among the serotype DQ 3 * 5 in control population (3.0%) compared with the serotype DQ 3 * 5 in Barrett's population (11.4%) but significant association was not found. Additionally, a similar difference in frequency of (8.2%) exists between the serotype DQ 3 * 6 in control population (13.9%) compared with the serotype DQ 3 * 6 in Barrett's population (5.7%) but significant association was not found.

A similar expression pattern is also reflected in the allelotype expression pattern presented in Table 26. The allelotype expression at this locus shows that the serotype DQ 2 in Barrett's population (34.1%) and the serotype DQ 3 in Barrett's population (33.5%) to be the most commonly expressed from all the other serotypes and from all types of population tested. However, comparing the serotype DQ 2 in Barrett's population (34.1%) and DQ 3 in Barrett's population (33.5%) with the serotype DQ 2 (30.5%) and DQ 3 (28.5%) in the control population the difference in frequency is very low and significant association was not found. The serotype DQ 6 in Barrett's population (16.5%) compared with the serotype DQ 6 in the control population (26.0%) presents a difference in frequency of (9.5%) and significant association was found between these two ($Ch^2 = 5.2740$, P=0.0252).

TABLE 25. Distribution and relative frequency (%) of DQβ1 genotypes in normal individuals and in total Barrett's oesophagus individuals.

Predicted DQβ1 serotype**	Distribution and Relative frequency (%)	
	Control* population	Total Barrett's oesophagus
	n (%)	n (%)
2 * 2	8 (7.9)	9 (10.2)
2 * 3	21 (20.7)	22 (11.4)
2 * 4	2 (2.0)	0 (0.0)
2 * 5	8 (7.9)	9 (10.2)
2 * 6	14 (13.9)	11 (12.5)
3 * 3	9 (8.9)	10 (11.4)
3 * 4	1 (1.0)	2 (2.3)
3 * 5	3 (3.0)	10 (11.4)
3 * 6	14 (13.9)	5 (5.7)
4 * 5	2 (2.0)	0 (0.0)
4 * 6	0 (0.0)	0 (0.0)
5 * 5	3 (3.0)	1 (1.1)
5 * 6	7 (6.9)	5 (5.7)
6 * 6	9 (8.9)	4 (4.5)

n= number of subjects

Total Barrett's n = 88

* Obtained from the west of Scotland tissue typing laboratory, Glasgow Royal Infirmary.

**The DQβ1 genotype and serological relationship is presented in Table 2.

TABLE 26. Total distribution and relative frequency (%) of DQβ1 genotypes in normal individuals and in total Barrett's oesophagus individuals.

Predicted DQβ1 serotype**	Total genotypic distribution and relative frequency (%)	
	Control* population	Total Barrett's oesophagus
	n (%)	n (%)
2	61 (30.5)	60 (34.1)
3	56 (28.5)	59 (33.5)
4	5 (2.0)	2 (1.1)
5	26 (13.0)	26 (14.8)
6	53 (26.0)♣	29 (16.5)♣

n= number of alleles

Total Barrett's n = 176

* Obtained from the west of Scotland tissue typing laboratory, Glasgow Royal Infirmary.

**The DQβ1 genotype and serological relationship is presented in Table 2.

♣ Significant difference between test group and controls ($P < 0.05$)

($Ch^2 = 5.2740$, $P=0.0252$).

Comparison of the HSP 70-2 genotypes in total Barrett's oesophagus patient population with the control population

The results at this locus are presented in Table 27. Among the entire Barrett's population the heterozygous genotype 1 * 2 (44.6%) presents the highest expresion. Also the results show that the heterozygous genotype 1 * 2 is by far the most commonly expressed in the control population (54.1%).

Comparing the heterozygous genotype 1 * 2 (44.6%) in Barrett's population with the heterozygous genotype 1 * 2 in the control population (54.1%) the difference in frequency is very small and significant association was not found. Additionally, comparing the homozygous genotype 1 * 1 in control population (11.7%) with the homozygous genotype 1 * 1 in Barrett's population (21.4%) the data reveals a difference in frequency of (9.7%) but again significant association was not found.

Unfortunately, this expression pattern is reflected in the allelotype expression pattern presented in Table 28. The allelotype expression at this locus shows that the allelotype 2* (936bp) in control population (61.3%) to be the most commonly expressed from all the other allelotypes and from all types of population tested. Comparing the allelotypes 1*(1117bp) in Barrett's population (42.9%) and 2*(936bp) in Barrett's population (57.1%) with the same allelotypes in the control population the difference in frequency is low and significant association was not found.

TABLE 27. Distribution and relative frequency (%) of HSP 70-2 genotypes in normal individuals and in total Barrett's oesophagus individuals.

HSP 70-2 genotypes	Distribution and Relative frequency (%)	
	Control population n (%)	Total Barrett's oesophagus n (%)
1 * 1	13 (11.7)	12 (21.4)
1 * 2	60 (54.1)	25 (44.6)
2 * 2	38 (34.2)	20 (35.7)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n= number of subjects

Total Barrett's n =56

TABLE 28. Total distribution and relative frequency (%) of HSP 70-2 allelotypes in normal individuals and in total Barrett's oesophagus individuals.

HSP 70-2 allelotypes	Total allelotypic distribution and relative frequency (%)	
	Control population	Total Barrett's oesophagus
	n (%)	n (%)
1*	86 (38.7)	48 (42.9)
2*	136 (61.3)	64 (57.1)

Allele 1* = 1117 bp

Allele 2* = 936 bp

n= number of alleles

Total Barrett's n =112

Comparison of the D6S273 microsatellite genotypes in total Barrett's oesophagus patient population with the control population

The results at this locus are presented in Table 29. Among the entire Barrett's population the heterozygous genotype 132-134 (20%) presents the highest expresion. Also the results show that the heterozygous genotype 132-134 is by far the most commonly expressed in control population (12.4%).

Comparing the heterozygous genotype 132-134 (20%) in Barrett's population with the heterozygous genotype 132-134 in the control population (12.4%) the difference in frequency is low (7.6%) and significant association was not found.

Similarly, comparing the homozygous genotype 132-132 in control population (11.4%) with the homozygous genotype 132-132 in Barrett's population (4.4%) the data reveals a difference in frequency of (7%) and significant association was not found.

This expression pattern is reflected in the allelotype expression pattern presented as in Table 30. The allelotype expression at this locus shows that the allelotype 134 in Barrett's population (33.3%) to be the most commonly expressed from all the other allelotypes and from all types of population tested. Additionally the allelotype 132 in the control population (28.1%) is the second most expressed between the two populations. Comparing the allelotype 132 in the control population (28.1%) with the allelotype 132 in the (26.7%) Barrett's population no great difference in frequency is present and significant association was not found. Similarly the frequency of the allelotype 134 in the control population (26.7.1%) compared with the allelotype 134 in the (33.3%) Barrett's population presents no great difference and significant association was not found. Additionally all the rest of the frequencies (Table 30) if compared with each other between the two populations, present similar or equal distribution and significant association were not found.

TABLE 29. Distribution and relative frequency (%) of D6S273 genotypes in normal individuals and in total Barrett's oesophagus individuals.

D6S273 genotypes	Distribution and Relative frequency (%)	
	Control population	Total Barrett's oesophagus
	n (%)	n (%)
126 – 126	2 (1.9)	1 (2.2)
126 – 128	1(0.9)	0 (0.0)
126 – 130	3 (2.9)	0 (0.0)
126 – 132	3 (2.9)	0 (0.0)
126 – 134	2 (1.9)	3 (6.7)
126 – 136	1(0.9)	0 (0.0)
126 – 138	0 (0.0)	1 (2.2)
128 – 128	3 (2.9)	0 (0.0)
128 – 130	5 (4.8)	0 (0.0)
128 – 132	3 (2.9)	1 (2.2)
128 – 134	0 (0.0)	1 (2.2)
128 – 136	0 (0.0)	1 (2.2)
128 – 138	0 (0.0)	0 (0.0)
130 – 130	3 (2.9)	1 (2.2)
130 – 132	3 (2.9)	4 (8.9)
130 – 134	6 (5.7)	5 (11.1)
130 – 136	3 (2.9)	0 (0.0)

132 – 132	12 (11.4)	2 (4.4)
132 – 134	13 (12.4)	9 (20.0)
132 – 136	4 (3.8)	1 (2.2)
132 – 138	9 (8.6)	5 (11.1)
134 – 134	10 (9.5)	4 (8.9)
134 – 136	5 (4.8)	2 (4.4)
134 – 138	10 (9.5)	2 (4.4)
136 – 136	2 (1.9)	0 (0.0)
136 – 138	2 (1.9)	1 (2.2)
138 – 138	0 (0.0)	1 (2.2)

n = number of subjects

Total Barrett's n =45

TABLE 30. Total distribution and relative frequency (%) of D6S273 allelotypes in normal individuals and in total Barrett's oesophagus individuals.

D6S273 allelotypes	Total allelotypic distribution and relative frequency (%)	
	Control population n (%)	Barrett's oesophagus n (%)
126	14 (6.7)	6 (6.7)
128	15 (7.1)	3 (3.3)
130	26 (12.4)	11 (12.2)
132	59 (28.1)	24 (26.7)
134	56 (26.7)	30 (33.3)
136	19 (9.0)	5 (5.6)
138	21 (10.0)	11 (12.2)

n = number of alleles

Total Barrett's n =90

DISCUSSION

DISCUSSION

Polymerase chain reaction (PCR) determination of DNA markers

The Polymerase chain reaction is a quick method of amplifying a region of DNA within which a RFLP and /or microsatellite site lie. The effectiveness of the technique is very much dependant upon the optimisation of the reaction conditions. However, standard PCR based commercial protocols are available for certain types of laboratory tests such as the Dynal *All*set™ SSP DQ "Low resolution sets" for tissue typing that was employed for the initial marker (DQβ1) in this study.

Value of RFLP and microsatellite markers analysis in studies involving immuno-relevant genes

In this study the restriction length polymorphism (RFLP), HSP 70-2 marker was also employed. The presence or absence of a restriction endonuclease cleavage site within a region of DNA gives rise, on digestion with that enzyme, to a possible biallelic pattern. The haplotype tends to be inherited in a Mendelian fashion allowing the inheritance of genes to be monitored. However, the main disadvantage is the low degree of information from a biallelic site. This is where the use of microsatellite polymorphism has revolutionised the field of DNA analysis. Microsatellites involve the presence of dinucleotide or less frequently tri- nucleotide sequences, tandem arranged and each allele is defined by the variation of repetitions of the sequence. Their high abundance throughout the genome and the large amount of information that is possible to determine, makes them ideal candidates for studying allelic variation in genes or loci of interest such as the D6S273 locus in this study.

Genotype and allelotype (predicted serotypes) data at the DQβ1 locus

In the recent past years tissue typing has started to be employed as a standard procedure in many types of diseases. Because of the localisation of the HLA DQβ1 genes to within the MHC class-I and class- II genes and the biological roles of the gene products, it is worth considering the expression patterns of the HLA molecules in tumours. Although some attempts have been made in the past years to find an association of gastric cancer with HLA, only poor associations have been reported. Many of these attempts where serologically based, producing non-specific allelic data. Recently, it was mentioned that the HLA class II gene DQβ1*0301 was strongly associated with gastric cancer in Caucasians (Lee *et al.*, 1996), which is not inconsistent with previous serological reports. However, similar recent molecular studies in Japan present no association of DQβ1*0301 with gastric cancer in Japanese (Ohmori *et al.*, 1997). Additionally no attempts have been made to consider HLA DQ- typing in Barrett's oesophagus condition in Caucasians and to compare such data with a gastric cancer data at the molecular level as we do in this study.

Because a DQβ1 "low resolution" protocol was employed it is more appropriate to present and discuss the results for this locus as a group of allelotypes i.e. predicted serotypes. The exact alleles incorporated in each serotype can be seen in Table 2. In our study the results for the DQβ1locus generally suggest that the heterozygous DQ 2 * 3 genotype is over expressed among all the test populations tested and the controls. This means that the heterozygous DQ 2 * 3 genotype may be of an importance. However, in the benign Barrett's oesophagus population the DQ 2 * 3 presents the highest overexpression, where if compared with the control a (11.7%) difference in expresion is present but significant association was not found for this genotype. This means that DQ 2 * 3 is not involved in the initial stages for the transformation of the normal oesophageal epithelium towards pre-malignant conditions. Although a more specific analyses (DQ "High resolution" typing) is probably needed for this serotype in order to identify exact allelic

specificities. Other serotypes such as the heterozygous DQ 3 * 6 and homozygous DQ 3 * 3 are present a secondary character of overexpression, which is means that such secondary serotypes could be supportive secondary elements in the transformation process from a normal to a pre-malignant condition in Barrett's oesophagus. However significant association was not found. Therefore such genotypic results lead us to the conclusion that the DQ 2 * 3, DQ 3 * 6 and DQ 3 * 3 are not significantly involved in Barrett's oesophagus.

The allelotypic (single serotype) expression of this locus presents that the serotype DQ 3 is the most commonly expressed in gastric cancer where if compared with the controls a difference of (11.3%) is present where significant association was found (Ch^2 =4.133, P=0.0421) This means that this allelotype (single serotype) DQ 3 is probably a significant factor that may be involved in the process of gastric carcinogenesis. The serotype DQ 3 among to the all the alleles that incorporates (Table 2), it incorporates the allele *0301. This means that our results probably are reproducible to those of (Lee *et al.*, 1996) but a certain prediction is not possible at this point unless a fine DQβ1 high resolution typing is performed specifically for the only allele *0301.

With regards to the serotype DQ 2 in benign Barrett's population, the data presents that it is the second most expressed serotype among all. However, no significant difference is presented among the test populations and in controls and significant association was not found. This suggests that the serotype DQ 2 is not an important element in the early stages of development of Barrett's oesophagus or gastric cancer. The serotype DQ 6 in the control population presents the highest expresion compared with the three test populations. This means that the serotype DQ 6 in Barrett's oesophagus it may have protective properties to the host due to the fact that is probably mutated in the three test populations as the low difference in frequencies suggests. However, significant association was not found suggesting that this serotype probably is not involved in Barrett's oesophagus at all.

In summary our data for this locus suggests, that our results probably are reproducible of that of (Lee *et al.*, 1996) regarding susceptibility of the allele *0301 in gastric cancer but a certain prediction is not possible at this point unless a fine DQβ1 typing is performed specifically for the allele *0301. Additionally, that the genotypes (combination of serotypes) DQ 2 * 3, DQ 3 * 6 and DQ 3 * 3 are not significantly associated with Barrett's oesophagus at all. Also DQ 2 * 3 is not involved in the initial stages for the transformation of the normal oesophageal epithelium to pre-malignant. The heterozygous DQ 3 * 6 and homozygous DQ 3 * 3 are not secondary supportive elements in the transition from a normal to a pre-malignant condition in Barrett's oesophagus. The DQ 6, alleles (*06011-*0613) does not play a protective part in the development of Barrett's oesophagus whereas the precise identification of specific DQ 6 alleles is not possible with a DQ "low resolution" protocol. Finally the serotype DQ 2 present loss of expression among the malignant Barrett's, gastric and control populations and is not involved in the initial stages in susceptibility to Barrett's oesophagus or gastric cancer. It is unclear however whether the loss of HLA expresion is due to immune selection or a secondary event occurring during tumour progression.

Genotype and allelotype data at the HSP 70-2 locus

The interest in this locus stemmed from it's positioning between HLA DQβ1 and TNF loci and from its involvement in carcinogenesis. It is known that HSP expression induced during the process of cell death. Induction of HSP 70 induces an infiltrate of T cells, macrophages and predominately dendritic cells into the tumours as well as an intratumoral profile of Th1 cytokine expresion (INF-γ, TNF-α, and IL-12) and enhances immunogenicity via a T cell mediated mechanism. The protection conferred by HSP 70 is both tumor and cell specific. Additionally HSP 70 targets immature APCs to make them significantly more able to capture Ags (Todryk *et al.*, 1999). The HSP 70/ HSc70 bind to BAG-1 proteins that in turn interacts with the Bcl-2, Raf-1, and steroid hormone receptors,

Shiah-1 and hepatocyte growth factor (HGF) all known to be involved in cell growth and cell migration (Naishiro *et al.,* 1999). It has been demonstrated that the complex HSP 70/ HSc70 -BAG-1 has a novel function for cell migration in human gastric cancer cells possibly through cooperation with cytoskeletal proteins (Naishiro *et al.,* 1999). Moreover BAG-1 modulates the activities of p53 proteins and p53-inducible factor Siah-1, and steroid hormone receptors (all promoting cell growth and survival) where the HSP 70 family of molecular chaperones in most cases recognises and bound mutant p53 proteins (Fourie *et al.,* 1997) thus acting as tumour development protector. Additionally HSP 70 can deliver NF-κB, a key transcriptional regulator of inflammatory responses, by binding to the p50 subunit of NF-κB a direct to the cytoplasm and nucleus of cells. Then it can bind DNA specifically and activate Igκ expresion and TNFα (Fujihara *et al.,* 1999). Therefore HSP 70 can be used as a vehicle for intracytoplasmic and intranuclear delivery of proteins or DNA for modulation of gene expresion. The polymorphic site HSP 70-2 was examined in this study by RFLP technique. Further more little work has previously been done on this locus with regards to gastric cancer and Barrett's oesophagus.

The results of the genotype data of this locus, presents the heterozygous genotype 1 * 2 (1117bp-936bp) to be the most commonly expressed among all the other genotypes in all test populations and the control. This suggests in general terms that the heterozygous genotype 1 * 2 (1117bp-936bp) may have some importance. More specifically, the heterozygous genotype 1 * 2 (1117bp-936bp) presents a large difference in frequency of (31.9%) between the malignant Barrett's oesophagus and the gastric population but significant association was not found and thus this genotype is not specifically involved in either two types of cancer. A difference in frequency of (24.1%) is present between the malignant Barrett's oesophagus and the controls where significant association was found between these two populations (Ch^2 =5.468, P =0.0194). These suggest that the heterozygous genotype 1 * 2 (1117bp-936bp) is actually overexpressed gradually from normal conditions towards malignancy and cancer. Therefore the protective action of the

HSP 70-2 proteins in individuals with this genotype, as described above, is present in Barrett's oesophagus but not in gastric cancer.

With regards to all the homozygous genotypes, the most commonly expressed is the 2 * 2 (936bp-936bp) among all other genotypes. This means that the homozygous genotype is probably important. The homozygous genotype 2 * 2 (936bp-936bp) presents a difference of (16.2%) between the malignant Barrett's oesophagus and the benign Barrett's oesophagus. However, significant association was not found. This means that this genotype is overexpressed progressively from pre-malignant conditions towards malignancy in Barrett's oesophagus individuals but is not specifically involved in malignant Barrett's oesophagus. This suggests that the HSP 70 genes are influenced by other factors in this case. This progressive overexpression is not seen in gastric cancer individuals (gastric cancer 33.3% Vs 34.2% controls) that suggest the HSP 70-2 alleles are probably mutated and the protective motif is lost. However significant association was not found, which suggests that again HSP 70 genes are influenced by other factors in this case too.

The allelotypic expression at this locus presents a similar pattern. The allelotype 2* (936bp) is the most commonly expressed in all types of population tested. However, the frequency of the allelotype 2* (936bp) in gastric cancer compared with the control is not great and significant association was not found. Similarly the difference in frequency of the allelotype 2* (936bp) between the two Barrett's oesophagus and controls is not great and again significant association was not found. These findings suggests that the allelotype 2* (936bp) alone is not associated with gastric cancer individuals and/or Barrett's oesophagus individuals where the protective action of the HSP 70 proteins is probably lost.

In summary the heterozygous genotype 1 * 2 (1117bp-936bp) is actually overexpressed gradually from normal conditions towards malignancy and cancer. Therefore the protective action of the HSP 70 proteins as described above is present in Barrett's oesophagus but not in gastric cancer. Similarly the homozygous genotype 2 * 2

(936bp-936bp) is overexpressed progressively from a pre-malignant condition towards malignancy in Barrett's oesophagus individuals but is not specifically involved in malignant Barrett's oesophagus. This suggests that the HSP 70 genes are influenced by other factors in this case. This progressive overexpression is not seen in gastric cancer individuals that suggest the HSP 70-2 alleles are probably mutated and the protective motif is lost. However significant association was not found, which suggests that HSP 70 genes are influenced by other factors in this case too.

The allelotypic expression at this locus presents a similar pattern where the allelotype 2* (936bp) is not associated with gastric cancer individuals and/or Barrett's oesophagus individuals where the protective action of the HSP 70 proteins is probably lost.

Genotype and allelotype data at the D6S273 microsatellite locus

The interest in this locus stemmed from it's positioning between HLA DQβ1,HSP 70 and TNF loci and from its involvement in different types of diseases, including autoimmune diseases. The marker D6S273 has been reported to strongly being associated with autoimmune diseases such as Ankylosing Spondylitis (AS), as reported by Brown *et al.,* (1998) in the British population and in a Canadian population (Singal *et al.,* 1998) and that this marker is probably in linkage with other genes within the HLA class III region, such as the HSP 70 and with other regions on chromosome 6 (6 p21.3). Microsatellite D6S273 and DQ region was found to be involved in susceptibility and protection to IDDM in a Belgian population (Moghaddam *et al.,* 1998).

The results of the genotype data for this locus, presents the heterozygous genotype 132bp – 134 bp to be the most common expressed among all the other genotypes in all test populations and the control. This suggests that this genotype might be important in this study. The data demonstrates that the highest expresion of the heterozygous genotype 132bp – 134 bp is present in Barrett's oesophagus. This suggests that this genotype may have some responsibility for susceptibility to Barrett's oesophagus. The difference in

frequency between the malignant Barrett's oesophagus population and the control populations is quite elevated (15.7%) where significant association was found between these two populations (Ch^2 =4.510, P =0.0337) suggesting that this genotype presents susceptibility to the onset of malignant Barrett's oesophagus. The difference in frequency between the gastric cancer population and the control population is quite elevated (11.9%) but significant association was not found, suggesting that this genotype does not present susceptibility to the onset of gastric cancer. Also this data suggests that in both cancerous populations the genotype 132-134 is overexpressed where in control and in benign (pre-malignant) populations this genotype presents low expresion. Therefore the genotype 132-134 must some how be involved in susceptibility cancer and specifically to malignant Barrett's oesophagus.

This type of expresion pattern involving the alleles 132 and 134 is also reflected in the microsatellite allelotype expression. Generally the allelotype 134 is the most commonly expressed among all other types of allelotypes. This suggests that the allelotype 134 is probably responsible for susceptibility to malignancy. The data obtained show that the allelotype 134 presents higher frequency in pre-malignant and malignant conditions than in controls. However, these differences are not significantly among all the test populations and controls. The most important difference is present between the control and the gastric cancer population (11.2%) but significant association was not found, suggesting that the allelotype 134 is not responsible for susceptibility to solid tumours and in this case gastric cancer.

The allelotype 132 presents higher expresion in malignant Barrett's population if compared with the benign Barrett's population (difference 10.5%) and / or gastric cancer population (difference 10.8%) but not with the controls. Significant association was not found among the test populations and the control. This suggests that the allele 132 is probably not responsible for susceptibility to solid tumours and in this case with malignant Barrett's carcinogenesis. The difference in frequency of the control population with the

gastric cancer population is (9.1%) and significant association was not found. This suggests that this allele probably is not responsible for susceptibility to solid tumours and in this case with gastric carcinogenesis. Additionally it can be concluded from our data that the allele 132 is not involved in the late stages of tumourogenesis (solid tumour stage) in gastric and Barrett's oesophageal cancer. Also according to the data obtained the difference in frequency between the control and benign Barrett's populations (8.9%) suggests that the allele 132 is also not involved in the initial steps for the onset of such cancers.

In summary among all the allelotypes tested the allelotypes 132 and 134 appears not to be susceptible in gastric cancer. Additionally the data show that in both cancerous populations the genotype 132-134 is overexpressed where in control and in benign (pre-malignant) populations this genotype presents lower expresion. The genotype 132-134 was found to present significant association with malignant Barrett's oesophagus (Ch^2 =4.510, P =0.0337) this finding leads to the conclusion that the heterozygous genotype 132-134 is associated with malignant Barrett's oesophagus.

HLA DQβ1 predicted serotype expresion is not associated with TNFa microsatellites

The localisation of the TNFa microsatellite locus between the MHC class-II and class-I genes and the importance of the biological roles of the gene products, is considered when looking at expression patterns of the HLA Class-II molecules in tumours and associations between these antigens and the TNFa alleles, in gastric cancer and in Barrett's malignant and benign oesophagus conditions. HLA Class-II (DP, DR, DQ) molecules present a non-constitutive expresion in most cell types, but physiological inducers such as TNF and INFγ can induce it. The patterns of expresion of such cell surface molecules changes in relation to cell function. A decrease or even complete lost of expresion are often associated with carcinogenesis. Furthermore, there seems to be a general lack of information available on this front.

We were able to carry out HLA DQβ1 typing of the three test populations in this study. The HLA-B locus is near the TNFa locus and strong linkage would therefore be expected, although a wide distribution is present. This means that the HLA-B locus is not in linkage with the TNFa locus. The data presents the higher frequency among all of the HLA DQβ1– TNFa alleles to be concentrated at DQ2-TNFa2 genotype in all populations tested. However, the difference in the frequencies of DQ2-TNFa2 genotype among the test populations is extremely low and significant association was not found, indicating that no association is present with progression of tumourogenesis.

In summary the results at the HLA DQβ1-TNFa loci obtained indicate a wide distribution of the DQβ1 alleles compared with the TNFa alleles. This suggest that the DQβ1 locus is not in linkage with the TNFa locus and therefore the TNFa locus is an independent factor for susceptibility to Barrett's oesophagus and in gastric cancer. These discoveries support the previously reported findings regarding TNFa from our research group in GIT carcinogenesis.

HSP 70-2 allelotype expresion is not associated with TNFa microsatellites

These two polymorphic sites are in close approximation to each other. The idea therefore to examine the possibility that alleles within these two loci may be in linkage was reasonable. Is known that induction of HSP 70 induces an infiltrate of T cells, into the tumours as well as an intratumoral profile of Th1 cytokine expresion including TNF and enhances immunogenicity via a T cell mediated mechanism that leads to the protection against tumor development and progression to aggressive tumour stages. Further more, there seems to be a general lack of information available on this front.

The data for such comparisons presents that the highest frequencies are concentrated in the allelotype combination 1*(1117bp)-TNFa2 (23.1%). However, the difference in the frequencies of 1*(1117bp)-TNFa2 genotype among the test populations is extremely low (malignant Barrett's 21.7% Vs gastric 20%) and significant association was

not found, indicating that no association is present with progression of tumourogenesis. Similarly the data for such comparisons presents that the allelotype combination 2*(936bp)-TNFa11 is the second most expressed from all the other genotypes but again the difference in the frequencies of 2*(936bp)-TNFa11 genotype among the test populations is extremely low and again significant association was not found, indicating that no association is present with progression of tumourogenesis. In addition the 2*(936bp)-TNFa2 genotype present an increased expresion in malignant Barrett's oesophagus population (20.0%) compared with the other two test populations (benign Barrett's 9.6% Vs gastric 14.2%) but the difference in frequency is not significantly great and significant association was not found. These finding suggest that 2*(936bp)-TNFa2 genotype is not associated with tumour progression.

In summary the results at the HSP 70-2 – TNFa loci obtained indicate a wide distribution of the HSP70-2 alleles compared with the TNFa alleles. It is well established that TNFa is in tight linkage disequilibrium with the HLA class II region - however they may have additive or separate effects on disease susceptibility. Again these discoveries support previously findings with regards to TNFa within our research group in GIT carcinogenesis.

HSP 70-2 allelotype expresion is associated with HLA DQβ1 predicted serotype expresion

Similarly, these two polymorphic sites share close proximity. The idea therefore to examine the possibility that alleles within these two loci may be in linkage with each other was reasonable. The importance of the biological roles of the gene products of such molecules was to be studied to consider expression patterns of the HLA Class-II (DQβ1) alleles in tumours and associations between these antigens and the HSP 70-2 alleles in gastric cancer and in Barrett's malignant and benign oesophagus conditions. Again, there is little information available specifically on this subject.

The data obtained indicates that the genotype of allele 2*(936bp)-DQ 3 presents the most common expresion from all the other genotypes and among all populations, which means that it might be associated with progression of tumourogenesis. However the results indicate that the differences in frequency among the three test populations for this genotype are low and significant association was not found, which is means that the genotype 2*(936bp)-DQ 3 is not associated with any specific type of cancer tested in this study. Similarly our data indicates that although the expresion of 1*(1117bp)-DQ2 genotype presents a secondary increased character the differences in frequencies among the three test populations are low and significant association was not found. This means that the genotype 1*(1117bp)-DQ2 is not associated with any specific type of cancer tested in this study. The only difference that is probably respectable is among 2*(936bp)-DQ 2 in malignant Barrett's oesophagus and in gastric cancer (difference of 12.3%) where significant association was found (Ch^2 =3.884, P=0.0487). This finding suggests that this genotype is associated with susceptibility to malignant Barrett's oesophagus and / or gastric cancer.

In summary the results at the HSP 70-2-DQβ1 loci obtained indicate a wide distribution of the HSP70-2 alleles compared with the DQβ1alleles. However, the only genotype that is associated with susceptibility to malignant Barrett's oesophagus and / or gastric cancer is 2*(936bp)-DQ 2. This suggests that the HSP70-2 locus is in linkage with the DQβ1locus only for the former genotype.

D6S273 microsatellite expresion is not associated with HLA DQβ1 predicted serotype expresion

These two polymorphic sites are in close proximity to each other. The idea therefore to examine the possibility that alleles within these two loci may be in linkage was reasonable. Although this marker it has been reported to be involved in different types of diseases and to be in linkage with the DR locus. D6S273 microsatellite alleles were found

to contribute to the genetic risk of IDDM-1, especially the alleles 136 and 140. However, a recombination linkage between DQ and TNFa was reported as an additional contributory factor in IDDM-1 (Moghaddam *et al.*, (1998). Additionally, the HLA DR and TNFβ markers have been reported by Haines *et al.*, (1998) to have strong genetic linkage with D6S273 marker and the onset of Multiple Sclerosis (MS). In other diseases such that of Rheumatoid arthritis (RA) D6S273 is now believed to have an association. Rheumatoid arthritis (RA) is a chronic inflammatory disease that is associated with the HLA-DRB1 genes. A resent study by Signal, D.P. (1998) revealed that two D6S273 microsatellite alleles 132 and 138 were significantly associated in Canadian patients with RA. Such studies suggest that D6S273 has not an independent association with diseases but probably are in strong linkage with other markers within the HLA. D6S273 marker now believed to be linked with some types of cancer. An example is that of Mazurenko *et al.*, (1999) in cervical cancer where demonstrated that LOH was observed in the HLA region within the marker D6S273 due to deletions of alleles.

The results obtained in our study indicate that the genotype DQ 2-132 in malignant Barrett's oesophagus (12.5%) present the highest expresion among the rest test populations for this genotype (benign 7.7%, gastric 3.1%). However the difference in frequencies is not great and significant association was not found. This suggests that this genotype do not contribute to the genetic risk of malignant Barrett's oesophagus. On the other hand the genotype DQ 2-134 in benign Barrett's oesophagus (15.4%) present the highest expresion among the rest test populations for this genotype (malignant 4.7%, gastric 3.1%). A difference in frequency of (12.3%) is present between the benign and the gastric population but significant associations were not found. This suggests that this genotype do not contribute to the genetic risk of benign Barrett's oesophagus and that is probably not involved at the initial stages of carcinogenesis. Additionally the results obtained in our study indicate that the genotype DQ 3-134 in gastric cancer (18.7%) present the highest expresion among the rest test populations for this genotype (benign 3.8% Vs malignant

15.6%). A difference in frequency of (14.9%) is present between the benign and the gastric population but significant association was not found. This suggests that this genotype do not contribute to the genetic risk of gastric cancer. Also the results indicate that the genotype DQ 3-138 in gastric cancer (12.5%) present the highest expresion among the rest test populations for this genotype (benign 3.8%, malignant 4.7%). However, the difference in frequencies and the fact that significant associations were not found, suggests that this genotype do not contribute to the genetic risk of gastric cancer.

In summary the results at the D6S273-DQβ1 loci obtained indicate that the DQ 2-134 do not contribute to the genetic risk of malignant Barrett's oesophagus. Also that the DQ 2 -132 genotype do not contribute to the genetic risk of benign Barrett's oesophagus and that is not involved at the initial stages of carcinogenesis. Also that the DQ 3-134 do not contribute to the genetic risk of gastric cancer and that the DQ 3-138 genotype do not contribute to the genetic risk of gastric cancer too. All these lead to the conclusion that the D6S273 locus is not in linkage with the DQβ1locus.

HSP 70-2 allelotype expresion is not associated with D6S273 microsatellite expresion

As already mentioned, certain alleles present linkage within the MHC in chromosome 6. The two loci of interest at this point are the HSP 70-2 locus compared with the D6S273 microsatellite locus looking for linkage between them. Further more, there seems to be a general lack of information available on this front.

The data obtained indicates that the genotypes 2*(936bp)-130, 2*(936bp)-132 2*(936bp)-134 present the most reasonable differences among the three test populations and the controls. More specifically the genotype 2*(936bp)-130 presents the highest frequency (9.1%) in benign Barrett's population when compared with the other two test populations studied (malignant 5.4% Vs gastric 1.6%) and the control (7.1%) but the difference in the frequencies are not significantly high among them and significant association was not found. This means that this genotype do not contribute to the genetic

risk of benign Barrett's oesophagus and that is probably has a protective motif. On the other hand the genotype 2*(936bp)-132 presents higher frequency (17.9%) in malignant Barrett's oesophagus where if compared with the benign Barrett's (4.5%) the difference in frequency present is significantly high (13.4%) but not if compared with the controls (19.2%) where significant association was not found. This means that this genotype is not associated with malignant Barrett's oesophagus. The difference in frequency between the benign Barrett's (4.5%) and the controls (19.2%) is significantly elevated (14.1%) but significant association was not found. Despite the fact that no association was found, this means that the genotype 2*(936bp)-132 is probably mutated in benign Barrett's oesophagus leading to low expresion and therefore the protective motif is lost. This also suggests that the genotype 2*(936bp)-132 is probably involved in the initial stages of the progression towards malignancy and cancer. Finally the genotype 2*(936bp)-134 presents no significantly high difference in frequency among the three populations studied and the control and significant association was not found. This means that this genotype does not contribute to the genetic susceptibility of any type of cancer tested in this study.

In summary, our findings suggest that none of the genotypes 2*(936bp)-130, 2*(936bp)-132 and 2*(936bp)-134 contribute to the genetic susceptibility of any type of cancer tested.

HLA DQβ1 predicted serotype expresion is associated with Barrett's oesophagus in total patient population compared with the controls

In order to assess the possibility whether certain genotypes and allelotypes (predicted serotypes) for this locus, in Barrett's population may present differences in frequency with the control population, a comparison was performed considering the two different types of Barrett's population (benign and malignant) as one. The data obtained was then compared with the control population.

The results obtained in our study indicate that the heterozygous genotype DQ 2 * 6 (presented as serotype) is the most commonly expressed in Barrett's population. However comparing this genotype with the same genotype in the control population the difference in frequency is not significantly different and significant association was not found. This finding suggests that this genotype is not involved in the genetic susceptibility to Barrett's oesophagus development. Similarly the heterozygous genotype DQ 2 * 3 present high expression in control population. However, comparing this genotype with the same genotype in the Barrett's population the difference in frequency is quiet high but not significantly different and significant association was not found. This finding suggests that this genotype is not involved in the genetic susceptibility to Barrett's oesophagus development. Additionally the heterozygous genotype DQ 3 * 5 present high expression in control population. However comparing this genotype with the same genotype in the Barrett's population the difference in frequency is quite high but not significantly different and again significant association was not found. This finding suggests that this genotype is not involved in the genetic susceptibility to Barrett's oesophagus development. Additionally the heterozygous genotype DQ 3 * 6 present high expression in control population. However comparing this genotype with the same genotype in the Barrett's population the difference in frequency is high but not significantly different and significant association was not found. This finding suggests that this genotype is not involved in the genetic susceptibility to Barrett's oesophagus development.

A similar expresion pattern is reflected in the allelotype (presented as serotype) DQ 2 and DQ 3 where these are the most expressed allelotypes in Barrett's population. However when these allelotypes compared with the same allelotypes in the control population the difference is not great and significant association was not found. This means that neither of these two allelotypes is associated in the genetic susceptibility to Barrett's oesophagus. Finally the allelotype DQ 6 presents quite high expresion in Barrett's oesophagus but if compared with the control the difference in frequency is not

high. Although significant association was found, (Ch^2 = 5.2740, P=0.0252, this finding suggests that this genotype is associated with genetic susceptibility to Barrett's oesophagus development.

In conclusion, our findings suggest that only one of the above allelotypes the DQ 6 (presented as serotype) is associated with genetic susceptibility to Barrett's oesophagus. Neither of the genotypes tested above are found to be associated with gastric cancer or Barrett's oesophagus development.

HSP 70-2 genotype expresion is not associated with Barrett's oesophagus in total patient population compared with the controls

Again in order to assess the possibility whether certain genotypes and allelotypes for this locus, in Barrett's population may present differences in frequency with the control population, a comparison was performed considering the two different types of Barrett's population (benign and malignant) as one. The data obtained was then compared with the control population.

The results obtained in our study indicate that the heterozygous genotype 1 * 2 is the most commonly expressed in Barrett's population. However comparing this genotype with the same genotype in the control population the difference in frequency is not significantly different and significant association was not found. This finding suggests that this genotype does not involved in the genetic susceptibility to Barrett's oesophagus development. Similarly the homozygous genotype 1 * 1 presents high expression in Barrett's population. However, comparing this genotype with the same genotype in the control population the difference in frequency is quiet high but significant association was not found. This finding suggests that this genotype is not associated with the genetic susceptibility to Barrett's oesophagus development.

This pattern is not reflected in the allelotype expresion. The most commonly expressed among all is the allelotype 2*(936bp) in control population. However compared

with the 2*(936bp) in Barrett's population no significant difference was present and significant association was not found. This means that this allelotype is not associated with the genetic susceptibility to Barrett's oesophagus development. The same pattern is present for the allelotype 1*(1117bp) which is found not to be associated with Barrett's oesophagus development.

In conclusion, our findings suggest that no one of the above genotypes and allelotypes tested associated with the genetic susceptibility of Barrett's oesophagus.

D6S273 microsatellite expresion is not associated with Barrett's oesophagus in total patient population compared with the controls

Finally in order to assess the possibility whether certain genotypes and allelotypes for this locus, in Barrett's population may present differences in frequency with the control population, a comparison was performed considering the two different types of Barrett's population (benign and malignant) as one. The data obtained was then compared with the control population.

The results obtained in our study indicate that the heterozygous genotype 132 * 134 is the most commonly expressed in Barrett's population. However comparing this genotype with the same genotype in the control population the difference in frequency is not significantly different and significant association was not found. This finding suggests that this genotype does not involved in the genetic susceptibility to Barrett's oesophagus development. Similarly the homozygous genotype 132 * 132 presents high expression in control population. However, comparing this genotype with the same genotype in the Barrett's population the difference in frequency is quite high but not significantly different and significant association was not found. This finding suggests that this genotype does not involved in the genetic susceptibility to Barrett's oesophagus development.

A similar expresion pattern is reflected in the allelotype expresion where the allelotypes 132 and 134 in between the two populations are the most expressed allelotypes

among all. However when these allelotypes are compared with each other between the two populations, the difference in frequency is not great and significant association was not found. This means that neither of these two allelotypes is associated with the genetic susceptibility to Barrett's oesophagus.

In conclusion, our findings suggest that no one of the above genotypes and allelotypes tested associated with the genetic susceptibility of Barrett's oesophagus.

Conclusions and prospects

Several points can be concluded from the work undertaken for this study.

Based on evidence suggesting involvement of genetic factors as risk factors for gastrointestinal cancers and that limited information is available regarding the precise role of various known genetic markers and genetic changes involved in gastrointestinal carcinogenesis, it is concluded that it was indeed very important to proceed in the determination whether the genetic alterations take place during gastric and oesophageal cancer development could be related with control of inflammation.

It can be concluded that allelotyping at the polymorphic sites DQβ1, HSP70-2 and D6S273 is possible by employing the optimised PCR-based techniques determined in this study.

Despite the wide distribution of allelotypes and genotypes among the populations tested for each marker it is concluded that significant associations were demonstrated among certain genotypes and allelotypes such as in the DQβ1 locus for the DQ 3 serotype (gastric cancer Vs controls; P = 0.0421), in the HSP 70-2 locus genotype 1 * 2 (malignant Barrett's Vs controls; P = 0.0194), in the D6S273 locus genotype 132-134 (malignant Barrett's Vs controls; P =0.0337) and the allelotype (serotype) DQ 6 (total Barrett's population Vs controls; P= 0.0252) suggesting their probable involvement in the control of inflammation in gastrointestinal cancers.

Also it can be concluded that significant association is present between certain allelotypes among certain loci where significant association was discovered between the DQβ1 locus and the HSP 70-2 locus (DQ 2 - HSP (936bp) alleles in gastric population Vs controls; P= 0.0487), suggesting their probable involvement in the control of inflammation in gastrointestinal cancers.

Additionally, it can be concluded that there is not a defined difference in genotype-allelotype distribution and no significant association was discovered among the three loci

tested compared with the TNFa 1-14 microsatellites. Therefore TNF locus is a primary independent locus for susceptibility to Barrett's oesophagus and probably gastric cancer.

Finally our study leads to the conclusion that the HLA-DQβ1 and HSP-70-2 loci are in linkage with each other for the genotype (DQ 2 - HSP (936bp) in gastric cancer.

The encouraging results that were obtained in this project could lead to the development of new immunogenetic therapeutic procedures for Barrett's oesophagus and/or gastric cancer, based on these specific immunogenetic targets (i.e. HLA-DQβ1, HSP-70-2, and D6S273) their protein products and relative cytokines. With further research it may be possible to use the data from this project to study such specific markers in greater depth. These results will assist the exploration of novel therapies in order to individualise treatment, in relation to specific histological types, depth of invasion of gastroesophagial cancers and probable reduction of inflammation. Combination of novel and new therapeutic modalities will assist to develop an accurate tailoring to the need of patients with gastroesophagial cancers and could minimise the side effects of current treatment. The fact that our markers are associated with gastrointestinal cancers, as supported by our data, could also encourage further study of other similar markers within close proximity to the loci investigated in our study. However, TNFa remains the main genetic component for such gastroesophagial cancers and the potential is there to use such multifactorial cytokines in the treatment of malignant diseases. Not only will it be possible to use this information for therapy but also within research in molecular diagnostics. In the future we will be able to diagnose specific cancers in the early stages, using diagnostic techniques based on such individual markers. Better treatment and obviously prognosis relies upon early and precise diagnosis and better understanding of the characterisation and progression of individual cancers.

REFERENCES

REFERENCES

Aaltonen, L. M., Partanen, J., Auvine, E., Rihkanen,H. (1999). " HLA DQ alleles and human papiloma virus DNA in adult-onset laryngeal papillomamatosis." The Journal of Infectious Diseases, **179,** 682 685

Aaltonen, L. A., Peltomaki, P., Leach, F. S., *et al.* (1993). " Clues to the pathogenesis of familial colorectal cancer. " Science, **260,** 812-816

Abdelatif, O.M.A., Chandler, F. W., Mills, L. R., *et al.* (1991). " Differential expression of c-myc and H-ras oncogenes in Barrett's epithelium: using colorimetric in situ hybridisation." Arch. Pathol. Lab. Med., **115,** 880-885

Adachi, M., Sekiya, M., Torigoe, T., Takayama, S., Reed, J. C., Miyazaki, T., *et al.* (1996). " Intereukin-2 up regulates BAG-1 gene expression through serine-rich region within IL-2 receptor beta c chain." Blood, **88,** 4118-4123

Aggarwal, B. B., Eessalu, T. E., and Hass, P. E. (1985). " Characterization of receptors for human necrosis factor and their regulation by γ-interferon." Nature, **318,** 665-670

Aihara, M., Tsuchimoto, D., Takizawa, H., Azuma, A., Wakebe, H., Ohmoto, Y., *et al.,* (1997). "Mechanisms involved in Helicobacter pylori-induced Interleukin-8 production by a gastric cancer cell line, MKN-45." Infection & Immunity, **65** (8), 3218-3224

Aikawa, T., Kojima, M., Onishi, H., Tamura, R., Fucuda, S., Suszuki, T., *et al.* (1996). " HLA DRB1 and DQB1 alleles and haplotypes influencing the progression of Hepatitis C." Journal of Medical Virology, **49,** 274-278

Ahn, S. G., Jeong, S.Y., Rhim, H., Kim, I. K. (1998). " The role of c-Myc and heat shock protein 70 in human hepatocarcinoma Hep3B cells during apoptosis induced by prostaglandin A_2 / Δ_{12}-prostaglandin J_2. Biochimica *et* Biophysica Acta, **1448,** 115-125

Al-Kasspooles, M., Moore, J. H., Orringer, M. B., *et al.* (1993). "Amplification and overexpression of the EGFR and erbB-2 genes in human oesophageal adenocarcinoma." Int. J. Cancer, **54,** 213-219

Aldener-Canava, A. & Olerup, O. (1994). "HLA-DQB1 " Low resolution" typing by PCR amplification with sequence-specific primers (PCR-SSP). Eur. J. of Immunogenetics, **24,** 447-455.

Asti, M., Martinetti, M., Zavaglia, C., Cuccia, M. C., Gusberti, L., Tinelli, C. (1999). " Human leukocyte antigen class II and III alleles and severity of Hepatitis C virus related chronic liver disease." Hepatology, **29** (4), 1272-1279

Atherton, J. C. (1998). "*H.Pylori* virulence factors." British Medical Bulletin, **54** (1), 105-120

Baggiolini, M., Dewald, B., Moser, B. (1994). " Interleukin-8 and related chemotactic cytokines – C-X-C and C-C chemokines." Adv. Immunology, **55,** 97-179

Barrett, N. R. (1950). " Chronic peptic ulcer of the oesophagus and oesophagitis." British Journal of Surgery, **38,** 175-82

Barleta, C., Scillato, F., Sega, F. M., Mannella, E. (1993). " Genetic alteration in gastrointestinal cancer. A molecular and cytogenetic study." Anticancer Res., **13** (6A), 2325-9

Bateman, A. C., Turner, S. J., Theaker, J. M., & Howell, W. M., (1998). " HLA-DQB1*0303 and *0301 alleles influence susceptibility to and prognosis in cutaneous malignant melanoma in the British Caucasian population." Tissue Antigens, **52,** 67-73

Beales, I. L. P., Post, L., Calam, J., Yamada, T. & Delvale, J. (1996). " Tumor necrosis factor alpha stimulates gastrin release from canine and human antral G cells: possible mechanism of the *Helicobacter pylori*-gastrin link." European Journal of Clinical Investigation, **26,** 609-611

Beutler, B., Cerami, A. (1989). " The biology of Cachectin / TNF a primary mediator of the host response." Ann. Rev. Immunology, **7,** 625-55

Blot, W. J., Devesa, S. S., Kneller, R. W., & Fraumeni, J. F. (1991). " Rising incidence of adenocarcinoma of the oesophagus and gastric cardia." JAMA, **265,** 1287-9

Blount, P.L., Ramel, S, Raskind, W. H., *et al.* (1991). " 17p Allelic deletions and P53 protein overexpression in Barrett's adenocarcinoma." Cancer Res., **51,** 5482-5486

Bodger, K., Wyatt, J, I., Heatley, R, V. (1997). " Gastric mucosal secretion of interleukin-10: relations to histopathology, *H. pylori* status and TNF-a secretion." Gut, **40,** 739-44

Bodmer, J. G., Marsh, S. G. E., Albert, E. D., Bodmer, W. F., Bontrop, R. E., ***et al.*** (1995). "Nomenclature for factors of the HLA system, 1995." Tissue Antigens, **46,** 1-18

Bodmer, W. F. (1994). "Cancer genetics." British Medical Bulletin, **50** (3), 517-526

Bouma, G., Crusius, J. B. A., Pool, O. M., Kolkman, J. J., Von Blomberg, B. M. E., Kostense, *et.al.* (1996). " Secretion of tumor necrosis factor a and lymphotoxin a in relation to polymorphism in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel diseases." Scand. J. Immunol., **43,** 456-463

Bronner, C. E., Baker, S. M., Morrison, P. T., *et al.* (1994). " Mutations in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer." Nature, **368,** 258-261

Brown, M. A., Pile, K. D., Kennedy, G., Campbell, D., Andrew, L., *et al.* (1998). " A genome – wide screen for susceptibility loci in Ankylosing Spondylitis." Arthritis & Rheumatism, **41** (4), 588-595

Bytzer, P., Christensen, P. B., Damkier, P., Vinding, K. & Seersholm. (1999). "Adenocarcinoma of the oesophagus and Barrett's oesophagus: A Population-Based study." The American Journal of Gastroenterology, **94** (1), 86-91

Carroll, M. C., Katzaman, P., Alicot, E. M., Koller, B. H., Geraghty, D. E., *et al.* (1987). " Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes." Proc., Nat., Acad., Sci., USA, **84,** 8535-8539

Charron, D., Lotteau, V., Tumel, P. (1984). " Hybrid HLA DC antigens provide molecular evidence for gene trascomplementation." Nature, **312,** 157

Christiansen, O.B., Pedersen, B., Mathiesen, O., Hustth, M. & Grunnet, N. (1996). " Maternal HLA class II alleles predispose to pregnancy losses in Danish women with recurrent spontaneous abortions and their female relatives." American Journal of Reproductive Immunology, **35,** 239-244

Cooper, C. S., Blair, D. G., Oskarsson, M. K., Tainsky, M. A., Eader, L. A., & Vande Wounde, G. F. (1984)." Characterization of human transforming genes from chemically transformed teratocarcinomas and pancreatic carcinoma cell lines." Cancer Res., **44** (1), 1-10

Correa, P. (1989). "In *Campylobacter* and Gastroduodenal disease." Blackwell Scientific, oxford, 139-145

Correa, P., Chen, V. W. (1994). " Gastric cancer." Cancer Survival, **19** (20), 55-76

Crabtree, J. E., Shallcross, T. M., Heathley, R. V., Wyatt, J. I., (1991). " Mucosal tumour necrosis factor-alpha and interleukin-6 in patients with helicobacter pylori-associated gastritis." Gut, **44,** 768-71

Curtsinger, J. M., Hilden, J. M., Cairns, J. S., Bach, F. H. (1987). " Evolutionary and genetic implications of sequence variation in two nonallelic HLA DR β chain cDNA sequences." Proc. Nat. Acad. Sci. USA., **84,** 209-213

Dearden, S. P., Taylor, G. M., Gokhale, D. A., Robinson, M. D., Thomson, W., Ollier, W., *et al.* (1996). " Molecular analysis of HLA-DQB1 alleles in childhood common acute lymphoblastic leukaemia." British Journal of Cancer, **73,** 603-609

D'Elios, M. M., Manghetti, M., De Carli, M., *et al.,* (1997). " T helper 1 effector cells specific for helicobacter pylori in the gastric antrum of patients with peptic ulcer disease." J. Immunol., **158,** 962-7

Dixon, M. F., Genta, R. M., Yardey, J. H., Correa, P. (1998). " Classification and grading of gastritis: the updated Sydney system." Am. J. Surg. Pathol., **20,** 1161-81

Dworniczak, B., Mirault, M. (1987). " Structure and expresion of a human gene coding for 71- KD heat shock cognate protein." Nucleic Acids Res., **15**, 5181-5197

Easton, D. F. (1994). " The inherited component of cancer." British Medical Bulletin, **50** (3), 527-535

Eberl, T., Amberger, A., Herold, M., Hengster, P., Steurer, W., *et al.* (1999). " Expresion of stress proteins, adhesion molecules and interleukin-8 in endothelial cells after preservation and reoxygenation." Cryobiology, **38,** 106-118

Eberl, T., Amberger, A., Hengster, P., Steurer, W., Windschwendtner, M., *et al.* (1999). "Expresion of heat shock proteins 60 and 70 is a marker of preservation-induced endothelial cell activation." Transplantation Proceedings, **31,** 1034-1036

Ehrenfried, J. A., Herron, B. E., Townsend Jr, C. M. & Evers, B. M. (1995). " Heat shock proteins are differentially expressed in human gastrointestinal cancer." Surgical Oncology, **4,** 197-203

Emtestam, L., Wallberg, P., Aldener, A. & Olerup, O. (1996). " Multiple basal cell carcinomas: no association with HLA-DRB, HLA-DQA1 or HLA-DQB1 in Swedish patients." British Journal of Dermatology, **134,** 886-891

Ernst, P. (1999). " Review article: the role of inflammation in the pathogenesis of gastric cancer." Alimentary Pharmacological Therapy, **13** (suppl.1), 13-18

Fan, X. J., Crowe, S. E., Behar, S., *et al.* (1998). " The effect of class II MHC expresion an adherence of Helicobacter pylori and induction of apoptosis in gastric epithelial cells: a mechanism for T H1 cell-mediated damage. J. Exp. Med., **(187),** 1659-69

Farthing, M. J. G. (1998). "*Helicobacter Pylori* infection: an overview." British Medical Bulletin, **54** (1), 1-6

Feder, J. N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D. A., Bassava, A., *et al.* (1996). " A novel MHC class-I like gene is mutated in patients with hereditary hemochromatosis." Nature Genetics, **13,** 399-408,

Feldman, R. A., James, A., Eccerslay, P. & Hardie, M. (1998). " Epidemiology of *Helicobacter Pylori*: acquisition, transmission, population prevalence and disease-to-infection ratio." British Medical Bulletin, **54** (1), 39-51

Ferrera, A., Olivo, A., Alaez, C., Melchers, W.J.G., Gorodzesky. (1999). " HLADQA1 and DQB1 Loci in Honduran women with cervical dysplasia and invasive cervical carcinoma and their relationship to human papillomavirus infection." Human Biology, **71** (3), 367-379

Filipe, M. I., Jankowski, J. (1993). " Growth factors and oncogenes in Barrett's oesophagus and gastric cancer cells." Endoscopy, **25,** 637-641

Firestein, G. S., Manning, A. M. (1999). " Signal transduction and transcription factors in Rheumatic Disease." Arthritis & Rheumatism, **42** (4), 609-621

Fishel, R., Lescoe, M. K., Rao, M. R., *et al.* (1993). " The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer." Cell, **75,** 1027-1038

Forman, D. (1998). "*Helicobacter Pylori* infection and cancer." British Medical Bulletin, **54** (1), 71-78

Fourie, A. M., Hupp, T. R., Lanes, D. P., Sang, B. C., Barbosa, M. S., *et al.* (1997). " HSP 70 binding sites in the tumor suppressor protein p53*." The Journal of Biological Chemistry, **272** (31), 19471-19479

Fox, J.G., Correa, P., Taylor, N.S., Zavala, D., Pantham, E., Janney, F., *et al.* (1989). "Campylobacter pylori-associated gastritis and immune response in a population at increased risk of gastric carcinoma." Am J Gastroenterol., **84** (7), 775-81.

Fraile, A., Nieto, A., Mataràn, L., Martin, J. (1998). " HSP 70 gene polymorphism in Ankylosing Spondylitis." Tissue Antigens, **51,** 382-385

Gallagher, G., Lindemann, M., Oh, H-H., Ferencik, S., *et al.* (1997). "Association of the TNFa2 microsatellite allele with the presence of colorectal cancer." Tissue Antigens, **50,** 47-51

Gallin, J. I., Goldstein, I. M. & Snyderman, R. (1992). " Inflammation overview." Inflammation: Basic principles and clinical correlates. **2nd ed.**, 1-4

Giordano, S., Ponzetto, C., Di Renzo, M. F., Cooper, C. S., Comiglio, P. M. (1989). " Tyrosine kinase receptor indistinguishable from the c-met protein." Nature (London), **339,** 155-156

Goldfeld, A, E., Delgano, J. C., Thim, S., Bozon, M. V., Uglialoro, A. M., Turbay, D., *et al.* (1998). " Association of an HLA-DQ allele with clinical Tuberculosis." JAMA, **279** (3), 226-228

Gray, A. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarret, J. A., *et al.* (1984). " Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity." Nature, **312,** 721-724

Gregoire, L., Lawrence, D.W., Kukuruga, D., Eisenbrey, A. B., Lancaster, W. D. (1994). " Association between HLA DQB1 alleles and risk for cervical cancer in African – American women." Int. J. Cancer, **57,** 504-507

Gregresen, P. K. (1989). " Biology of disease: HLA class II polymorphism: Implications for genetic susceptibility to autoimmune disease." Laboratory Investigation, **61** (1), 5-19

Grubic, Z., Moghaddam, P. H., Giphart, M. J., Žunec, R., Èeèuk-Jelièiè and kaštelan, A. (1999). " Distribution of alleles at two microsatellite loci (D6S273 and TNFa) in Croatian population." Coll. Antropol., **23** (1), 87-90

Gulley, M. L., Pulitzer, D. R., Eagan, P. A., Schneider, B. G. (1996). " Epstein-Barr virus interferon is an early event in gastric carcinogenesis and is independent of bcl-2 expression and p53 accumulation." Hum. Pathol., **27** (1), 20-7

Gustafsson, K., Wiman, K., Emmoth, E., Larhammar, D., Bohme, J. *et al.* (1984). "Mutations and selection in the generation of class II histocompatibility antigen polymorphism." EMBO J., **3,** 1655-1660

Goustin, A. S., Leof, E. B., Shipley, G. D., *et al.* (1986). " Growth factors and cancer." Cancer Res., **46,** 1015-1029

Haas, J.P., Kimura, A., Truckenbrodt, H., Suschke, J.,Sasazuki, T., Volgger, A., Albert, E. D. (1995) " Early –onset pauciarticular juvenile chronic arthritis is associated with a mutation in the Y-box of the HLA– DQA1 promoter." Tissue Antigens, **45,** 317

Haines, J. L., Terwedow, H. A., Burgess, K., Pericak-Vance, M. A., Rimmler, J. B., *et al.* (1998). " Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity." Human Molecular Genetics, **7** (8), 1229-1234

Hamada, H., Seidman, M., Howard, B. H., Gorman, C. M. (1984). " Enhanced gene Expression by the poly (dT-dG) poly (dC-dA) sequence." Mol Cell Biol., **4,** 2622-2630

Hameeteman, W., Tytgat, G. N. J., Houthoff, H. J., van den Tweel, J. G. (1989). " Barrett's oesophagus: development of dysplasia and adenocarcinoma." Gastroenterology, **96**, 1249-1256

Haranaka, K., Satomi, N., and sakurai, A. (1984). " Differences in tumour necrosis factor productive ability among rodents." Brit. J. Cancer, **50,** 471-478

Harrison, G. S., Drabkin, H. A., Kao, F. T., Hartz, J. Hart, I. M., *et al.* (1987). " Chromosomal location of human genes encoding major heat shock protein HSP 70." Somatic cell & Molecular Genetics, **13** (2), 119-130

Hearne, C. M., Ghosh, S. & Todd, J. A. (1992). " Microsatellites for linkage analysis of genetic traits." TIG, **8** (8), 288-294

Heistercamp, N., Stephenson, J. R., Groffen, J., Hansen, P. I., de Klein, A., Bartram, C. R., Grosfeld, G. (1983). " Localization of the c-abl oncogene adjacent to a translocation break point in chronic myelocytic leukaemia." Nature (London), **306,** 239-242

Helland, A., Olsen, A. O., Gjoen, K., Akselsen, H. E., Sauer, T., Magnus, P., *et al.* (1998). "An increased risk of cervical cancer intra-epithelial neoplasia grade II-III among human papillomavirus positive patients with the HLA DQA1 *0102-DQB1 *0602 haplotype: A population based- case-control study in Norwegian women." Int. J. Cancer, **76,** 19-24

Helland, A., Borresen, A. L., Kristensen, G., Ronnigen, K. S. (1994). "DQA1 and DQB1 genes in patients with squamus cell carcinoma of the cervix: Relationship to human papillomavirus infection and prognosis." Cancer Epidemiology, Biomarkers& Prevention, **3,** 479-486

Henning, G., Behrens, J., Truss, M., Frisch, S., *et al.* (1995). " Progression of carcinoma cells is associated with alterations in chromatin structure and factor binding at the E-cadherin promotor in *vivo*." Oncogene, **11** (3), 475-484

Hiraki, A., Kaneshige, T., Kiura, K., Ueoka, H., Yamane, H., Tanaka, M., *et al.* (1999). " Loss of HLA haplotype in lung cancer cell lines: Implications for immunosurvaillance of altered HLA class I/ II phenotypes in lung cancer." Clinical Cancer Research, **5,** 933-936

Hiyama, E., Yokohama, T., Tatsumoto, N., Hiyama, K., Imamura, Y., et *al.* (1995). "Telomerase activity in gastric cancer." Cancer Res., **55** (15), 3258-3262

Hoffman, P. S. & Garduno, R.A. (1999). " Surface-associated heat shock proteins of *Legionella pneumophila* and *Helicobacter pylori*: Roles in pathogenesis and immunity." Infectious Diseases in Obstetrics and Gynecology, **7,** 58-63

Horii, A., Nakasuru, S., Miyoshi, Y., *et al.* (1992). " The APC gene responsible for familial adenomatus polyposis is mutated in human gastric cancer." Cancer Res., **52,** 3231-3233

Hongyo, T., Buzard, G. S., Palli, D., Weghorst, C. M., Ammorosi, A., *et al.* (1995). " Mutations of the K-ras and p53 genes in gasric adenocarcinomas from a high-incidence region around Florence, Italy." Cancer Res., **55** (12), 2665-72

Howell, W. M., Leung, S. T., Jones, D. B., Nakshabendi, I., Hall, M. A., Lunchbury, L. S., *et al.* (1995). " HLA-DRB, -DQA and -DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma." Human Immunology, **43,** 29-37

Hsieh, L. L., Huang, Y. C. (1994). " Loss of heterozygosity of APC / MCC gene in differentiated and undifferentiated gastric carcinoma in Taiwan." Cancer Lett., **96** (2), 169-74

Huang, Y., Meltzer, S. j., Yin, J., *et al.* (1993). " Altered messenger RNA and unique mutational profiles of P53 and Rb in human oesophageal carcinomas." Cancer Res., **53,** 1889-1894

Hunt, C., Morimoto, R. I. (1985). " Conserved features of eukaryotic HSP70 genes revealed by comparison with the nucleotide sequence of human HSP70." Proc. Nat. Acad. Sci. USA, **82,** 6455-6459

Hurley, C. K., Gregersen, P. K., Steeiner, N., Bell, J., Hartzamen, R., Nepom, G. (1988). "Polymorphism of the HLA-D region in American blacks. J. Immunol., **140,** 855,

Imazeki, F., Omata, M., Nose, H., *et al.* (1992). " P53 gene mutations in gastric and oesophageal cancers." Gastroenterology, **103,** 892-896

Irene, E., Bruisma, H., Viser, H., Mieke, J., Hazes, W., Ferdinard, C., *et al.* (1999). " HLA DQ associated predisposition to and dominant HLA-DR associated protection against rheumatoid arthritis." Human Immunology, **60,** 152-158

Jaattela, M. (1999). " Escaping Cell Death: Survival proteins in cancer." Experimental Cell Research, **248,** 30-43

Jackow, C. W., Mc Ham, J. B., Friss, A., Alvear, J., Reveille, J. R., Duvic, M. (1996). " HLA DR5 and DQB1*03 class II alleles are associated with cutaneous T-cell lymphoma." Journal of Investigative Dermatology, **107** (3), 373-376

Jacob, C. O., Mykytyn, K., & Tashman, N. (1993). " DNA polymorphism in cytokine genes based on length variation in simple-sequence tandem repeats." Immunogenetics, **38,** 251-257

Jacob, C. O. and MacDevitt, H. (1988). " Tumor necrosis factor -α in autoimmune lupus nephritis." Nature, **331,** 356-358

Jacob, C. O., Fronek, Z., Lewis, G., Koo, M., Hansen, J., and McDevitt, H. O. (1990). " Heritable MHC class II-associated differences in production of tumour necrosis factor α: relevance to the genetic predisposition to systemic lupus erythematosus." Proc Nat. Acad. Sci. USA, **87,** 1233-1238

Jonsson, A. K., Hyldig-Nielson, J. J., Servenies, B., Larhammar, D., Andersson, G., *et al.* (1987). " Class II genes of the human major histocompatibility complex. Comparisons of the DQ and DX α and β genes." J. Biol. Chem., **262,** 8767

Kageshita, T., Naruse, T., Hirai, S., Ono, T., Horikoshi, T., Nakagawa, H., *et al.* (1997). "Molecular genetic analysis of HLA class II alleles in Japanese patients with melanoma." Tissue Antigens, **49,** 466-470

Karttunen, R., Karttunen, T., Ekre H-Pt, MacDonald T. (1995). " Interferon gamma and interleukin-4 secreting cells in the gastric antrum in Helicobacter pylori positive and negative gastritis." Gut, **36,** 341-5

Kassai, K., Yoshikawa, T., Yoshida, N., Hashiramoto, A., Kondo, M., Murase, H. (1999). "*Helicobacter pylori* water extract induces Interleukin-8 production by gastric epithelial cells." Digestive Diseases and Sciences, **44** (2), 237-241

Kawanishi, K., Shiozaki, H., Doki, Y., Sakita, I., Inoue, M., Yano, M., *et al.* (1999). " Prognostic significance of heat shock proteins 27 and 70 in patients with squamus cell carcinoma of the oesophagus." Cancer, **85** (5), 1649-1657

King, R.J.B. (1996). Cancer Biology, 1-181

Koreth, J., O'Leary, J. J. & McGee, J. O'D. (1996). " Microsatellites and PCR genomic analysis." Journal of Pathology, **178,** 239-248

Kuniyasu, H., Yasui, W., Yokozaki, H., Akagi , M., Akama, Y., *et al.* (1994). " Frequent loss of heterozygosity of the long arm of chromosome 7 is closely associated with progression of human gastric carcinomas." Int. J. Cancer, **59** (5), 597-600

Kuniyasu, H., Yasui, W., Kitadai, Y., Yokozaki, H., Ito, H., Tahara, E. (1992). " Frequent amplifications of the c-met gene in scirrhous type stomach cancer." Biochem. Biophys. Res. Commun., **189** (1), 227-32

Kunkel, T. A. (1993). " Nucleotide repeats. Slippery DNA and diseases." Nature, **365,** 207-208

Kusher, D. I., Ware, C. F. and Gooding, L. R. (1990). " Induction of the heat shock response protects cells from lysis by necrosis factor." J. Immunology, **145** (9), 2925-31

Lauren, P. (1965). "The two histological main types of gastric carcinoma diffuse and so-called intestinal type carcinoma." Acta Pathol-Microbiol., **64,** 31- 49

Lauwers, G. Y., Scott, G, V., Hendricks, J. (1994). " Immunohistochemical evidence of aberrant bcl-2 protein expression in gastric epithelial dysplasia." Cancer, **73** (12), 2900-4

Leach, F. S., Nikolaides, N. C., Papadopulos, N., *et al.* (1993). " Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. " Cell, **75,** 1215-1225

Lee, J. E., Lowy, A. M., Thomson, W. A., Lu, M., Loflin, P. T., Skibber, J. M., *et al.* (1996). "Association of gastric adenocarcinoma with the HLA class II gene DQB1*0301." Gastroenterology, **111,** 426-432

Lee, J. E., Lu, M., Mansfield, P. F., Platsoucas, C. D., Reveille, J. D. & Ross, M. I. (1996). "Malignant melanoma: Relationship of the human leukocyte antigen class II gene DQB1*0301 to disease recurrence in American joint committee on cancer stage I and II." Cancer, **78** (4), 758-763

Lee, J. E., Reveille, J. D., Ross, M. I. & Platsoucas, C. D. (1994). " HLA-DQB1*0301 association with increased cutaneous melanoma risk." Int. J. Cancer, **59,** 510-513

Lee, C. W., Yang, H. Y., Kim, S. C., Jung, J. H., Hwang, J. J. (1998). " HLA class II allele associations in Korean patients with pemphigus." Dermatology, **179,** 349-352

Leunk, R. D., Johnson, P.T., David, B.C., *et al.* (1988). " Cytotoxic activity in broth culture filtrates of *Campylobacter pylori.*" J. Med. Microbiol., **26,** 93-9

Lin, L., Jin, A., Kimura, M., Carrington, M., & Mignot, E. (1997). " DQ microsatellite association studies in three ethnic groups." Tissue Antigens, **50,** 507-520

Lin, L., Wu, M. S., Shun, C. T., Lee, W. J., sheu, J. C., Wang, T. H. (1995). " Occurrence of microsatellite instability in gastric carcinoma is associated with enhanced expression of erbB-2 oncoprotein." Cancer Res., **55** (7), 1428-30

Lombardi, M. L, Mercuro, O., Pirozzi, G., Ionna, F., Lombari, V., *et al.* (1998). " Molecular analysis of HLA DRB1 polymorphism in Italian melanoma patients." Journal of Immunotherapy, **21** (6), 435-439

Lulli, P., Grammatico, P., Brioli, G., Catricalà, C., Mirellini, M., *et al.* (1997). " HLA-DR and DQ alleles in Italian patients with melanoma." Tissue Antigens, **51,** 276-280

Lynch, H. T., Lanspa, S. Smyrk, T., Boman, B., Watson, P., Lynch, P. (1991). " Hereditary non polyposis colorectal cancer syndromes I & II. Genetics, pathology, natural history and cancer control part 1." Cancer Genet. Cytogenet., **53,** 143-160

Mai, U. E. H, Perez-Perez, G. I., Wahl, L. M., *et al.,* (1991). "Soluble surface proteins from *Helicobacter pylori* activate monocytes / macrophages by lipopolysaccharide independent mechanisms." J. Clin. Invest., **87,** 894-900

Mann, A., Hanchard, B., Morgan, O. St. C., Wilks, R., Cranston, B., Nam, J., *et al.* (1998). "Human leukocyte antigen class II alleles associated with human T-cell lymphotropic virus type I infection and adult T-cell leukaemia / lymphoma in black population." Journal of National Cancer Institute, **90** (8), 617-622

Manthey, C. L., and Vogel, S. N. (1992). " The role of cytokines in host responses to endotoxins." Rev. Med. Microbiology, **3,** 72-79

Markowits, S., Wang, J., Myeroff. L., *et al.* (1995). " Inactivation of the type II TGF - βreceptor in colon cancer cells with microsatellite instability." Science, **268,** 1336-1338

Martin, P. M., Harding, A., Chadwick, R., Kronick, M., *et al.* (1998). " Characterization of 12 microsatellite loci of the human MCH in a panel of reference cell lines." Immunogenetics, **47**, 131-138

Martin, D. Z., Hughes, S. H., Barbacid, M., (1986). " A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences." Nature (London), **319,** 743-748

Mazurenko, N., Attaleb, M., Gritsko, T., Semjovnova, L., Pavlova, L., *et al.* (1999). " High resolution mapping of chromosome 6 deletions in cervical cancer." Oncology Reports, **6,** 859-863

McManus, R., Moloney, M., Borton, M., Finch, A., *et al.* (1996). " Association of Celiac disease with microsatellite polymorphisms close to the tumor necrosis factor genes." Human Immunology, **45,** 24-31

Milner, C. M., & Campbell, D. (1990). " Structure and expresion of the three MHC-linked HSP- 70 genes." Immunogenetics, **32,** 242-251

Melcher, A., Todryk, S., Hardwick, N., Ford, M., Jacobson, M. & Vile, R. (1998). " Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression." Nature Medicine, **4** (5), 581-587

Meltzer, S. J., Yin, J., Huang, Y., *et al.* (1991). " Reduction to homozygosity involving P53 in oesophageal cancers demonstrated by the polymerase chain reaction." Proc Natl Acad Sci USA, **88,** 4976-4980

Moghaddam, H. P., Zwinderman, A. H., De Knijf, P., Roep, O., *et al.* (1997). " TNFa microsatellite polymorphism modulates the risk of IDDM in Caucasians with the high –risk genotype HLA DQBA1*0501-DQB1*0201/ DQA1*0301-DQB1*0302." Diabetes, **46,** 1514 -1516

Moghaddam, H. P., De Knijf, P., Roep, B.O., Van der Auwera, B., Naipal, A., *et al.* (1998). "Genetic Structure of IDDM 1. Two separate regions in the Major Histocompatibility Complex Contribute to susceptibility or protection." Diabetes, **47,** 263-269

Montoya, L., Saiz, G., Rey, G., Vela, F., Clerici-Larradet, N. (1998). " Cervical carcinoma: human papilloma virus infection and HLA-associated risk factors in the Spanish population." European Journal of immunogenetics, **25,** 329-337

Moris, J. C. & Bruckner, H. W. (1997). " Gastric cancer: Molecular and cellular abnormalities." Encyclopedia of Cancer, **2,** 681-692

Muscarella, M., Rachlinski, M. K. & Bloom, S.E. (1998). " Expression of cell death regulatory genes and limited apoptosis induction in avian blastodermal cells." Molecular Reproduction and development, **51,** 130-142

Naishiro, Y., Adachi, M., Okuda, H., Yawata, A., Mitaka, T., Takayama, S., *et al.* (1999). " BAG-1accelerates cell motility of human gastric cancer cells." Oncogene, **18,** 3244-3251

Navaratnam, R. M. & Winslet, C. M. (1998). " Barrett's oesophagus." Postgraduate Med. J., **74,** 653-657

Nawa, A., Nishiyama, Y., Kobayashi, T., Wakahara, Y., Okamoto, T., Kikkawa, F., *et al.* (1995). "Association of human Leukocyte Antigen-B1*03 with cervical cancer in Japanese women aged 35 years and younger." Cancer, **75** (2), 518-521

Nedospasov, S. A., Udalova, I. A., Kuprash, D. Y., Turetskya, R. L. (1991). " DNA sequence polymorphism at the human tumor necrosis factor locus." J. Immunology, **147,** 1053-9

Nicolaides, N. C., Papadopoulos, N., Liu, B., *et al.* (1994). " Mutations at two PMS homologues in hereditary nonpolyposis colon cancer." Nature, **371,** 75-80

Nishimura , H., Emoto, M., kimoura, K., & Yoshikai, Y. (1997). " Hsp70 protect with *Salmonella Choleraesuis* against TNFa-induced cell death." Cell Stress & Chaperones, **2** (1), 50-59

Noach, L. A., Bosma, N, B., Jansen, J, Hoek, F. J.,van Deventer, S. J. H., Tygat, G. N. J. (1994). " Mucosal tumour necrosis factor alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection." Scand. J. Gastroenterology, **29,** 425-9

Odunsi, K., Terry, G., Ho, L., Bell, Cuzick, J., Ganesan, T. S. (1995). " Susceptibility to human papillomavirus-associated cervical intra-epithelial neoplasia is determined by specific HLA DR-DQ alleles." Int. J. Cancer, **67,** 595-602

Odunsi, K., Terry, G., Ho, L., Bell, Cuzick, J., Ganesan, T. S. (1995). "Association between HLA DQB1*03 and cervical cancer intra-epithelial neoplasia." Molecular Medicine, **1** (2), 161-171

Ohomori, M., Yasunaga, S., Maehara, Y., Sugimachi, K., & Sasazuki , T. (1997). " DNA typing of HLA class I (HLA-A) and class II genes (HLA-DR-DQ-DP) in Japanese patients with gastric cancer." Tissue Antigens, **50**, 277-282

Olerup, O., Aldener, & Fogdell, A. (1993). " HLA-DQB1 and DQA1 typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours." Tissue Antigens, **41,** 119-134

Peltomaki, P., Aaltonen, L. A., Sistonen, P., *et al.* (1993). " Genetic mapping of a locus predisposing to human colorectal cancer." Science, **260,** 810-812

Peltomaki, P., Lothe, R., Aaltonen, L. A., et al. (1993). " Microsatellite instability is associated with tumours that characterize the hereditary non-polyposis carcinoma syndrome." Cancer, Res., **53,** 5853-5855

Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Paladino, M. A., *et al.* (1984). " Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin." Nature, **312,** 724 -729

Petersdorf, E. W., Longton, G. M., Anasetti, C., Mickelson, E. M., Smith, A. G., Martin, P. J. & Hansen, J. A. (1996). " Definition of HLA-DQ as a transplantation antigen." Proc. Natl. Acad. Sci. USA, **93,** 15358-15363

Rao, D. V., Watson, K. & Jones, G. L. (1999). " Age-related attenuation in the expression of the major heat proteins in human peripheral lymphocytes." Mechanisms of ageing and development, **107,** 105-118

Reichstetter, S., Brünnler, G., Kalden, J. R. & Wassmuth, R. (1996). " DQB1 promoter variability and linkage in Caucasoids." Human Immunology, **51,** 73-80

Reinshagen, M., Loeliger, C., Kuehnl, P., Weiss, U., Manfras, B. J., Adler, G., Boehm, B. O. (1996). " HLA class II gene frequencies in Chron's disease a population based analysis in Germany." Gut, **38,** 538-542

Rhyu, M. G., Park, W. S., Jung, Y. J., Choi, S. W., Meltzer, S. J. (1994). "Allelic deletions of MCC / APC and p53 are frequent late events in human gastric carcinogenesis." Gastroenterology, **106** (6), 1584-8

Ribas, G., Neville, M., Wixon, J. L., Cheng, J., & Campbel, R. D. (1999). " Genes encoding three new members of the leukocyte antigen 6 superfamily and a novel member of Ig superfamily, together with genes encoding the regulatory nuclear chloride ion channel protein (hRNCC) and N^{00}-N^{00} – dimethylarginine dimethylaminohydrolase homologue, are found in 30-kb segment of the MHC class III region." The Journal of Immunology, **163,** 278-287

Ried, T., Heselmeyer-Haddad, K., Blegen, H., Schröck, E. & Auer, G. (1999). " Genomic changes defining the genesis, progression, and malignancy potential in solid tumors: A phenotype/genotype correlation." Genes, Chromosomes & Cancer, **25,** 195-204

Rigas, B. (1996). " HLA and disease association: The case of gastric cancer." Gastroenterology, **111** (2), 523-526

Roit, I. M., Brostoff, J., Male, D. K. (1996). " T-cell Receptors and MHC molecules." Immunology, **4th ed**, 5.2-5.10

Rood, M.J., van Krugsten, M. V., Zanelli, E., van Der Linden, M. W., Keijsers, V., Scheuder, G. M. T., *et al.* (2000). " TNF-308 and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus." Arthritis & Rheumatism, **43,** (1) 129-134

Roussomustakai, M., Satsangi, J., Welsh, K., Luis, E., Faning, G., targan, S., *et al.* (1997). "Genetic markers may predict disease behaviour in patients with ulcerative colitis." Gastroenterology, **112,** 1845-1853

Rowland, M. & Drumm, B. (1998). " Clinical significance of *Helicobacter* infection in children." British Medical Bulletin, **54** (1), 95-103

Rugge, M., Genta, R, M. (1999). " Epstein-Barr virus: a possible accomplice in gastric oncogenesis." J. Clin. Gastroenterology, **29** (1), 3-5

Rugge, M., Shiao, Y. H., Correa, P., Baffa, R., DiMario, F. (1992). " Immunohistochemical evidence of p53 overexpression in gastric epithelial dysplasia." Cancer Epidemiology Biomarkers Prev., **1** (7), 551-4

Saal, K., Vollmers, H. P., Muller, J., Kohler, J., Hohn, H., Muller-Hermelink, H. K. (1993). " Cytogenetic differences between intestinal and diffuse types of human gastric carcinoma." Virchows Arch. B cell pathol. Incl. Mol. Pathol., **64** (3), 145-50

She, J. X. (1996). " Susceptibility to type I diabetes: HLA-DQ and DR revisited". Immunol. Today, **17,** 329-336

Salotra, P., Chauhan, D., Ralhan, R. & Bhatnagar, R. (1995). " Tumour necrosis factor-alpha induces preferential expression of stress proteins in virulent promastigotes of *Leishmania donovani*." Immunology Letters, **44,** 1-5

Sanjeevi, C. B., Hjelmstrom, P., Hallmans, G., Wiklund, F., Lenner, P., Angstrom, T., Dillner, J. (1996). " Different HLA-DR-DQ haplotypes are associated with cervical intraepithelial neoplasia among human papillomavirus type-16 seropositive and seronegative Swedish women." Int. J. Cancer, **68,** 409-414

Sargent, C.A., Dunham, I., Trowsdale, J., & Campbell, D. R. (1989). " Human major histocompatibility complex contains genes for the major heat shock protein HSP70." Proc. Nat. Acad. Sci., **86,** 1968-1972

Sasajima, K., Kawachi, T., Matsukura, T., Samo N., Sugimura, J. (1979). "Intestinal metaplasia and adenocarcinoma induced in the stomach of rats by N-propyl-N'-nitro-N-nitrosoguanidine." J. Cancer res. Clin. Oncol., **94**, 201-206

Seder, R, A., Paul, W. E. (1994). "Acquisition of lymphokine-producing phenotype by CD4+ Tcells." Annu. Rev. Immunol., **12,** 635-73

Seeley R. R., Stephens T. D., Tate P. (1992). Anatomy and physiology. 2nd ED., 857-858

Sherwood L. (1993). Human physiology. 2nd ED., 503-505

Shigetada, T., Rokutan, K., Takahashi, M., Nikawa, T., and Kishi, K. (1996). " Induction of heat shock proteins and their possible roles in macrophages during activation by macrophage colony-stimulating factor." Biochem. J., **315,** 497-504

Singal, D. P., Li, J., Ye, M., & Lei, K. (1998). " D6S273 microsatellite polymorphism and susceptibility to rheumatoid arthritis." Tissue Antigens, **52,** 353-358

Slighotn, J. L., Blechl, A. E., Smithis, O. (1980). " Human Gγ and Aγ globin genes: complete nucleotide sequence suggests that DNA can be exchanged between these genes." Cell, **21**, 627-638

Stanbridge, E. J. (1990). "Identifying tumor suppressor genes in human colorectal cancer." Science, **247,** 12-13

Stemmerman, G., Heffelfinger, S. C., Noffsinger, A., Zhong Hui, Y., Milller, M. A. & Fenoglio-Preiser, C. M. (1994). " The molecular biology of oesophageal and gastric cancer their precursors: Oncogenes, tumor suppressor genes and growth factors." Human Pathology, **25** (10), 968-981

Stell, C. M. (1994). " Identification and characterisation of cancer genes." British Medical Bulletin, **50** (3), 536-559

Stevens, A., Lowe, J. (1995). "Neoplasia." Pathology, 34-56

Sud, R., Talbot, I, C., Delhanty, J. D. (1996). "Infrequent alterations of the APC and MCC genes in gastric cancers from British patients." Br. J. Cancer, **74** (7), 1104-8

Tahara, E., Kuniyasu, H., Yasui, W., Yokozaki, H. (1994). "Gene alterations in intestinal metaplasia and gastric cancer." Eur. J. Gastroenterol. Hepatol., **6, (**suppl.1), S97-102

Takayama, S., Krajewski, S., Krajewska, M., Kitada, S., Zapata, M., Kochel, K., *et al.* (1998). " Expression and location of HSP 70 / Hsc-Binding anti-apoptotic BAG-1 and its variants in normal tissue and tumor cell lines." Cancer Res., **58,** 3116-3131

Tamura, G., Maesawa, C., Suzuki, Y., Ogasawara, S., *et al.* (1993). " Primary gastric carcinoma cells frequently lose of heterozygosity at the APC and MCC genetic loci." Jpn. J. Cancer Res., **84** (10), 1015-8

Tanahasi, T., Kita, M., Kodama, T., Yamaoka, Y., Sawai, N., *et al.* (2000). " Cytokine expresion and production by purified Helicobacter pylori urease in human gastric epithelial cells." Infect. Immun., **68** (2), 664-71

Tarassi, K., Carthy, d., Papasteriades, C., Boki, K., Nikolopoulou, N., *et al.* (1998). " HLA-TNF haplotype heterogeneity in Greek SLE patients." Clinical & Experimental Rheumatology, **16,** 66-68

Taylor, G. M., Dearden, S., Payne, N., Ayres, M., Gokhale, D. A., Birch, J. M., *et al.* (1997). " Evidence that an HLA-DQB1 haplotype influences susceptibility to childhood common acute lymphoblastic leukaemia in boys provides further support for an infection-related aetiology." British Journal of Cancer, **78** (5), 561-565

Todryk, S., Melcher, A.A., Hardwick, N., Linardakis, E., Bateman, A., et al. (1999). " Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake." The Journal of Immunology, **163,** 1398-1408

Trowsdale, J., Young, J. A. T., Kelly, A.P., Austin, P. J., Carson, S., Meunier, H., *et al.* (1985). " Structure sequence and polymorphism in the HLA D region." Immunol. Rev., **85,** 5-10

Van der Auwera, B., Waeyenberge, C. V., Schuit, F., Heimberg, H., Vandewalle, C., Gorus, F., *et al.* (1995). " DRB1* 0301 protects against IDDM in Caucasian with the high risk heterozygous DQA1*0301-DQB1*0302 / DQA1*0501-DQB1*0201 genotype." Diabetes, **44,** 527-530

Van Den Boogert, J., Van Hillegersberg, R., De Bruin, R.W.F., Tilanus, H.W., Siersema, P. D. (1998). " Barrett's oesophagus: Pathophysiology, diagnosis and management." Scand. J. Gastroenterology, **33,** 449-453

Van Dekken, H., Geelen, E., Dingens, W. N. M., Wijnhoven, B. P. L., Tilanus, H. W., Tanke, H. J. & Rosenberg, C. (1999). " Comparative genomic hybridisation of cancer of the gastroesophagial junction: Deletion of 14Q31-32.1 Discriminates between oesophageal (Barrett's) and gastric cardia adenocarcinomas." Cancer Res., **59,** 748-752

Wei, Y., Zhao, X., Kariya, Y., Teshigawara, K., Uchida, A. (1995). " Inhibition of proliferation and induction of apoptosis by abrogation of heat-shock protein (HSP) 70 expression in tumor cells." Cancer immunol. Immunolother., **40,** 73-78

Weissmann, G. (1992). " Inflammation Historical Perspective. Inflammation: Basic principles and clinical correlates." **2nd ed.**, 5-9

Wooster, R., Cleton-Jansen, A. M., Collins, N., et al. (1994). " Instability of short tandem repeats (microsatellites) in human cancer." Nature, Gemet., **6,** 152-156

Wong, G. H. W., Elwell, J.H., Oberley, L. W., Goeddel, D. V. (1989). "Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumour necrosis factor." Cell, **58** (5),923-31

Wrigth, P. A. & Williams, G.T. (1993). " Molecular biology and gastric carcinoma." Gut, **34,** 145-147

Yamaoka, Y., Kita, M., Kodama, T., Sawai, N., Imanishi, J. (1996). "*Helicobacter pylori cag A* gene and expresion of cytokine messenger RNA in gastric mucosa." Gastroenterology, **110,** 1744 -1752

Yasuda, K., Shiraishi, N., Suemashu, T., Yamaguchi, K., Adachi, Y. & Kitano, S. (1999). "Rate of detection of lymph node metastasis is correlated with the depth of submucosal invasion in early stage gastric carcinoma." Cancer, **85** (10), 2119-2123

Yokozaki, H., Ito, R., Nakayama, H., Kuniyasu, H., Tanihama, K., Tahara, E. (1994). "Expresion of CD44 abnormal transcripts in human gastric carcinomas." Cancer Lett., **83** (1-2), 229-34

Yokozaki, H., Kuniyasu, H., Yasui, W., Tahara, E. (1994). " Genetic characteristics of scirrhous gastric carcinomas." Gan To Kagaku Ryoho, **21** (14), 2371-7

Younes, M., Lebovitz, R. M., Lechago, L.V., *et al.* (1993). " P53 protein accumulation in Barrett's metaplasia, dysplasia and adenocarcinoma: a follow up study." Gastroenterology, **105,** 1637-164

APENDIX

APENDIX-I

MATERIALS AND EQUIPMENT

Tris-base	Life Technologies™, UK
Boric acid ($H_3 BO_3$)	Life Technologies™, UK
Urea ($NH_2 CO NH_2$)	Life Technologies™, UK
Acrylamide (CH_2: $CH.NH_2$)	Life Technologies™, UK
Bis-Acrylamide (CH2: CH.CO.NH) $_2CH_2$)	Life Technologies™, UK
Ammonium Persulfate $(NH_4)_2S_2O_8$	Sigma® Chem. Co.USA
TEMED ($C_6H_{16}H_2$)	Sigma® Chem. Co.USA
Acetic acid glacial	Fisher scientific Ltd, UK
Dynal Allset™ SSP-DQ "Low resolution " sets	Dynal. Ltd, UK
Ampli-Taq™	Adv. Biotechnologies,UK
(10×) Ampli-Taq Buffer	Adv. Biotechnologies, UK
Cresol Red ($C_{21} H_{17} O_5 S Na$)	Sigma® Chem. Co.USA
Glycerol	W. Ransom & son Plc.UK
Liquid paraffin	Pinewood Labs, Ltd. UK
PCT – 225 DNA Engine Tetrad, 96-well thermal cycler, Version 3.0	MJ-Research. Inc. USA
Uno-thermoblock™, 96-well thermal cycler (no heated lid)	Biometra™ USA
Agarose SEA KEM LE™	FMC® Co. USA
Ultra Pure Agarose™	GIBCO™. BRL. UK
Ethidium Bromide	Sigma® Chem. Co.USA
Orange-G ($C_{16} H_{10} N_2 O_7 S_2 Na_2$)	Sigma® Chem. Co.USA
Pst-I Restriction Enzyme	Stratagene. Co. USA

(10×) Universal Buffer™	Stratagene. Co. USA
[α^{32}- P] d-CTP (350 MBq / ml-10mCi /ml)	Amersam Pharmacia biotech Ltd, UK
hyperLadder-I™	Bioline. Ltd., U.K.
ϕx174-RF DNA / Hαe III	GIBCO™. BRL. UK
Formamide (Ultra Pure™)	GIBCO™. BRL. UK
HSP-70 oligonucleotide primers	MWG-Biotech AG, UK
D6S273 oligonucleotide primers	MWG-Biotech AG, UK
100 bp DNA Ladder	GIBCO™. BRL. UK
EDTA	anaLAR. Co.UK