Genotype, phenotype and cancer: Role of low penetrance genes and environment in tumour susceptibility

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Role of heredity and lifestyle in sporadic cancers is well documented. Here we focus on the influence of low penetrance genes and habits, with emphasis on tobacco habit in causing head and neck cancers. Role of such gene-environment interaction can be well studied in individuals with multiple primary cancers. Thus such a biological model may elucidate that cancer causation is not solely due to genetic determinism but also significantly relies on lifestyle of the individual.

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1. Introduction

Cancer has been a scourge on the human population for many years. Although numerous advances have been made in prevention, diagnosis and treatment of the disease, it still continues to torment mankind. As is widely believed, cancer is the result of many genetic and epigenetic changes in a population of cells as well as in the surrounding stroma and blood vessels. These genetic alterations disrupt several molecular pathways in the cell and lead to self-sufficiency in growth signals, insensitivity to growth-control signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, invasion and metastasis (Hanahan and Weinberg 2000; Balmain *et al* 2003).

According to a census of human cancer genes, 291 cancer genes have been reported which is about 1% of the total human genes (Futreal *et al* 2004). Ninety percent of these genes show somatic mutations in cancer, 20% show germline mutations and 10% show both. The most common mutations which have been reported in cancer cells are chromosomal translocations which result in fusion proteins (e.g. bcr-abl fusion protein due to translocation of *abl* gene to *bcr* locus in Chronic Myeloid Leukemia) or apposition of one gene to the regulatory region of another gene (e.g. *RET* and *NTRK1* in thyroid papillary carcinoma), resulting in giving the cell a growth advantage. These chromosomal translocations are common in lymphomas, leukemias and mesenchymal tumours (Futreal *et al* 2004). The most common domain mutated in the cancer genes has been reported to be a protein kinase domain (Knudson 2002) which is an important enzyme in the cascade reaction in signal transduction (e.g. RET mutated in multiple endocrine neoplasia, *MET* in hereditary papillary renal carcinoma, *KIT* in hereditary gastrointestinal stromal tumour syndrome). Proteins coded by cancer genes often have growth stimulatory, DNA binding or transcriptional regulation activities. Almost in all cancers mutations in genes causing deregulated cell proliferation and suppressed cell death lead to tumour progression (Evan and Vousden 2001).

Keywords. Gene-environment interactions; low penetrance genes; multiple primary cancers; SNP

Abbreviations used: HNSCC, Head and neck squanous cell carcinoma; MMR, mismatch repair; *NAT*, N-acetyltransferase; SNP, single nucleotide polymorphism; UADT, upper aero-digestive tract; XME, Xenobiotic metabolizing enzyme.

2. Are cancers hereditary?

Cancers can be classified broadly as (i) hereditary cancers – these occur due to germline mutations in the tumour suppressor or proto-oncogenes and are transmitted from one generation to another; or (ii) sporadic cancers – these are cancers which are not transmitted from one generation to another. The vast majority of human cancers are sporadic and arise due to accumulation of multiple somatic mutations occurring spontaneously or from carcinogen exposure. Germline mutations in high penetrance (frequency with which the cancer phenotype manifests in the persons harbouring the germline mutation) genes such as *BRCA1* and 2 (breast and ovarian cancers), *APC* (colorectal cancers), *hMLH1/hMSH1* (hereditary non polyposis colon cancer), *RET* (medullary carcinoma thyroid) or TP53 (Li Fraumeni syndrome) result in hereditary cancers. Hereditary cancers are rare (less than 5% of all cancers). The high penetrance of the autosomal dominant inherited condition results in multiple cases of cancers among first and second degree relatives, generally at a younger age (e.g. BRCA1 gene mutation). Such mutations have been identified from cancer families using linkage analysis and positional cloning and have been well studied genotypically and phenotypically.

According to Knudson (2002), heritable predisposition to cancer is recognized for virtually every form of cancer including sporadic cancers. In the past decade studies on mice have shown that many genes with relatively small effects control cancer susceptibility. Also, analysis of genetic risk of cancer has shown that most non-hereditary, sporadic cancers develop in genetically predisposed individuals; this predisposition is the result of several low penetrance genes rather than single gene mutations (Imyanitov *et al* 2004; Houlston and Peto 2004). It has been observed from epidemiological studies that the first degree relatives of sporadic cancer patients have a 2–3-fold higher risk of developing cancer at the same site. Familial clustering observed in certain sporadic cancers without obvious Mendelian inheritance suggests that there is also a genetic component in addition to environmental factors (Peto and Houlston 2001). This could be explained on the basis that the family members with the similar genetic background, are exposed to the same environment including air-pollutants, food, infections, lifestyles, etc.

Etzel *et al* (2003) observed the evidence of familial aggregation of lung cancer among relatives of late-onset lung cancer cases. Also patients with sporadic cancers in the upper aero-digestive tract (UADT) are at a much higher risk of developing a second cancer in the same region due to repeated carcinogenic insult (Fujita *et al* 1998). In our multiple primary neoplasia (MPN) Registry (i.e. registry of patients with more than one primary cancer) at Tata Memorial Hospital, the commonest site of cancer is in the UADT. About 80% of the patients have tobacco habit, indicating a strong predisposition to tobacco-related cancers (unpublished data). Analysis of such data provide evidence that genetic predisposition may contribute to the development of a large proportion of non-familial cancers. Similar conclusions are drawn from the analysis of data from extensive twin studies which indicate that genetic predisposition accounts for as much as 40% of the individual risk for various sporadic cancers (Lichtenstein *et al* 2000; Paul *et al* 2000).

Significant advances have been made in identifying genes involved in cancer predisposition. Mutations in the gene *MUTYH* result in an autosomal recessive syndrome characterized by the development of multiple colorectal adenomas and cancer (Chow *et al* 2004). Mutations in DNA mismatch repair (MMR) genes cause hereditary nonpolyposis colorectal cancer (HNPCC), and MMR defects are associated with a significant proportion of sporadic cancers (Yang *et al* 2004). Taken together, all these data suggest that the genes responsible for sporadic cancers may be the low penetrance genes that may not confer Mendelian patterns of inheritance but may be involved in cancer predisposition.

3. Genes and environment

Since the 18th century it has been recognized that exposure to environmental chemicals plays a major role in the etiology of human cancers. Soot was found to be carcinogenic causing scrotal cancers in chimney sweepers (Doll 1975; Tomatis 1990). Since then it has been established that environmental factors play a dominant role in a majority of sporadic cancers (Bostwick *et al* 2004; Wogan *et al* 2004; Sugiyama 2004).

However, all individuals exposed to the same type and dose of carcinogen do not develop cancer. It is now understood that cancer development is not only due to exogenous or endogenous carcinogens but their interactions with genes that are involved in the detoxification of these carcinogens, repair of DNA damage and control of cell signalling and cell cycle. Due to carcinogen exposure, development of sporadic cancers may be facilitated by a cumulative effect of mutations or polymorphisms in these genes. Under this polygenic model, each allele confers a small genotypic risk which combine additively or multiplicatively to confer a range of susceptibilities (Houlston and Peto 2004). Until recently, no direct link between exposure to carcinogens, genetic alteration and human cancer could be drawn. Subsequently a large number of case-control studies have attempted to establish the role of polymorphisms in carcinogen metabolizing enzymes as well as other important genes involved in tumour susceptibility (Buch *et al* 2002; Aka *et al* 2004; Jhavar *et al* 2004; Slattery *et al* 2004). Genetic variations in a number

of critical regulatory pathways modify the tobacco-related cancer risk (Wu *et al* 2004). Genetic predisposition alone may not be responsible for causing cancer but a combination of susceptibility genes and exposures including environmental factors could contribute to the development of non-familial, sporadic cancers (figure 1). Life styles, such as smoking, alcohol consumption, use of hormones play a major role in increasing the risk of cancer.

4. Role of low penetrance genes in carcinogenesis

In the study of sporadic cancers, the focus of research has traditionally been on the exogenous and endogenous carcinogens and how they result in genetic alterations that result in cancer. However, it is being increasingly realized that genetic predisposition due to polymorphisms and mutations in the low penetrance genes also play an important role in determining the outcome of carcinogen exposure and the risk of cancer development (figures 1–3). The genes grouped as low penetrance genes have a major role to play in carcinogenesis and serve as markers for predicting cancer risk. The cells in the body are constantly being exposed to carcinogens from the microenvironment (superoxide anions, hydroxyl radicals and hydrogen) or macroenvironment (chemical carcinogens, viruses,

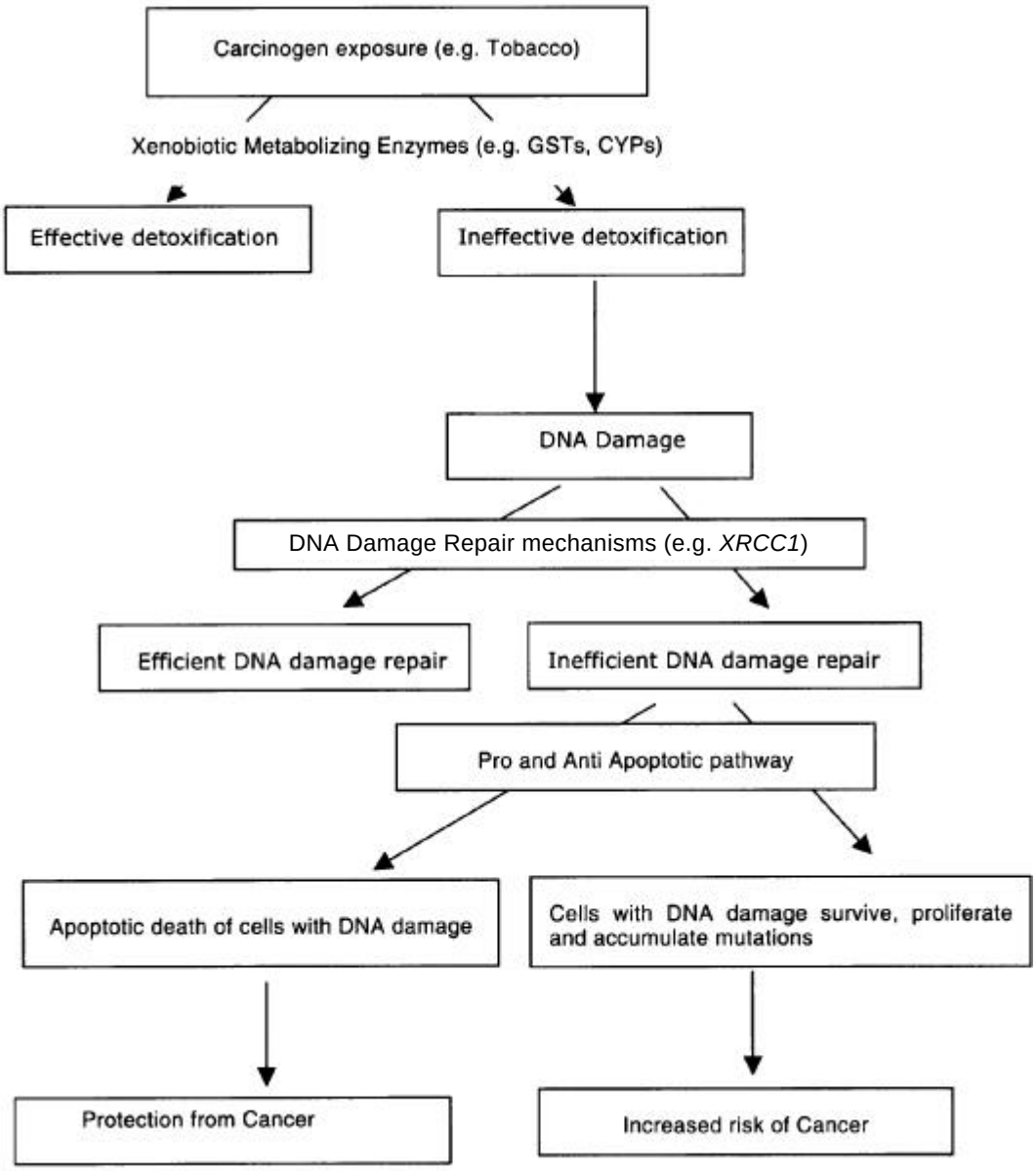

Figure 1. Multistep carcinogenesis model and the role of low penetrance genes.

radiation, etc.). The primary event due to carcinogen exposure is DNA damage which has to be safeguarded. A number of genes are responsible for carrying out this function and thereby preventing cancer. They are the probable cancer susceptibility genes.

The cancer susceptibility genes belong to one of three classes: gatekeepers, caretakers and landscapers (Kinzler and Vogelstein 1998). The inevitable DNA damage due to carcinogens can be prevented by the 'caretaker genes' which have a role in maintaining the integrity of the genome. There are two sets of enzymes which fall in this category – (i) enzymes that detoxify endogenous and exogenous carcinogens called xenobiotic metabolizing enzymes or, (ii) repair enzymes that repair the damage in the DNA from the carcinogens. If the damaged DNA is not efficiently repaired, the 'gate-keeper genes' which directly regulate growth and differentiation pathways of the cell and comprise of oncogenes and tumour-suppressor genes, either stop the cell from proliferating further in order to repair the damaged DNA once again, or eliminate the cell via programmed cell death (apoptosis). If the cell with damaged DNA continues to proliferate, accumulation of further genetic lesions may result in malignant transformation (figure 1).

It has also been proposed that altered stroma plays a role in epithelial cell growth which increases cancer susceptibility. This has been termed as the 'landscaper' effect (Kinzler and Vogelstein 1998). Barcellos-Hoff (2001) believe that normal cells effectively restrict malignant behaviour and that such forces must be conquered to establish a tumour. Disruption of the microenvironment promotes the transition from preneoplastic to neoplastic growth. Epithelial-mesenchymal interactions are known in normal development as well as in tumour progression (Bhowmick *et al* 2004; Shekhar *et al* 2003). It has been postulated that the lesions in the stroma (surrounding mesenchymal tissue) modulate the risk of epithelial malignancy: an abnormal stromal environment induces the overlying epithelium to progress to malignant transformation. Development of carcinoma of the epithelium was considered to be due to the epithelial cells being positioned in a highly abnormal microenvironment. Mutations in *SMAD4* gene have been associated with juvenile polyposis syndrome, which is a rare disorder in which the patients have polyps throughout the gastrointestinal tract. Loss of *SMAD4* was reported in the stromal region and hence supported the landscaper hypothesis. However, Woodford-Richens *et al* (2000) have reported that the loss of both alleles of *SMAD4* gene was seen in stroma as well as epithelium and so *SMAD4* probably acts as a 'gatekeeper' gene.

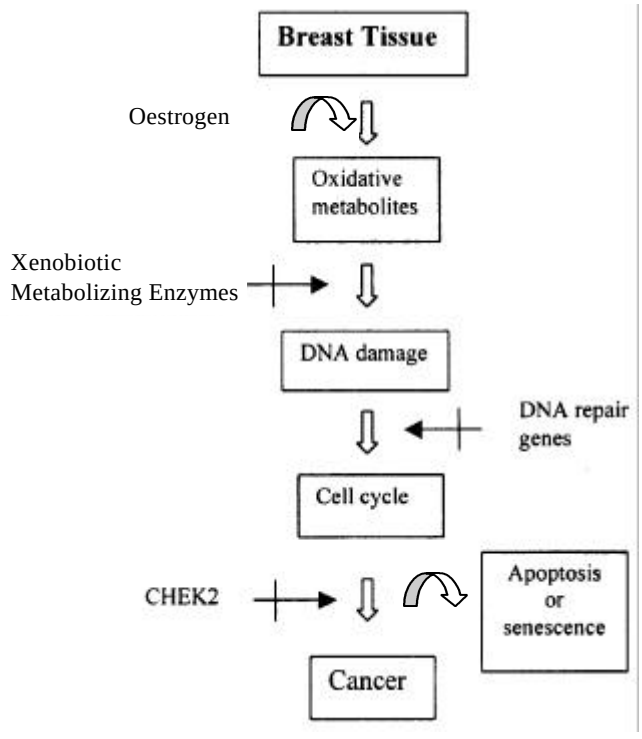

Figure 2. Genetic model for sporadic breast cancer.

5. Single nucleotide polymorphism and genetic susceptibility to cancer

With the sequencing of the human genome, it is evident that about 99·9% of the DNA is identical in every human genome (Lander *et al* 2001; Marth *et al* 2001; Venter *et al* 2001). The 0·1% difference is responsible for the inter-individual variation and the unique phenotype of each individual. These minor genetic variations, seen as single base change in the genome are known as single nucleotide polymorphisms (SNPs). SNPs play an important role in promoting susceptibility to diseases as well as the response of the individual to various drugs and environment/carcinogens (Hemminki and Shields 2002). A systematic survey of SNPs in the coding regions of human genes indicated about 50% of the SNPs bring about a change in the amino acid and in the remaining the change is non-conservative (Cargill *et al* 1999). Epidemiological studies aim at studying SNPs in candidate genes as risk factors.

Altered function of low penetrance genes due to SNPs may affect the gene-environment and gene-gene interaction, thereby increasing the risk of the development of sporadic cancers. It is believed that large scale genotyping of samples from cancer patients compared to normal, healthy individuals with similar exposures, will lead to important breakthroughs in understanding gene-environment and gene-gene interactions as mechanistic basis for

the common polygenic sporadic cancers. There is a large volume of literature available where polymorphisms in low penetrance genes and environmental factors, specially life styles, have been associated with increased risk of different sporadic cancers (Fearon 1997; Potter 1999).

6. Mechanism of action of low penetrance genes

Selection of low penetrance, cancer-susceptibility genes depends on the knowledge of the biochemical and physiological pathways that are known to be involved in the process of carcinogenesis. In order to protect ourselves against the deleterious effects of carcinogens present in the diet as well as in the environment the body has evolved a host of metabolic enzymes and other protective proteins. A cumulative effect of the xenobiotic metabolizing enzymes (XME), DNA repair enzymes and cell cycle check point proteins play a role in safeguarding the genome.

The XMEs are divided, on the basis of their metabolism, into phase I and phase II enzymes. Phase I enzymes metabolically activate procarcinogens to genotoxic electrophilic intermediates, and phase II enzymes conjugate the intermediates to water-soluble derivatives, thus completing the detoxification cycle. Many of the XMEs show polymorphisms which have been well characterized and are known to affect the enzyme activity. Some of the enzymes belonging to the Cytochrome P450 superfamily such as CYP1A1, CYP1B1 and those belonging to Glutathione S Transferase family such as GSTM1, GSTT1, GSTP1 as well as N-acetyltransferase (NAT) family are involved in oestrogen biosynthesis and conversion of oestrogen metabolites and therefore are associated with breast cancers (Mitrunen and Hirvonen 2003, van der Hel *et al* 2003). Similarly some of these enzymes involved in bioactivation/detoxification of tobacco carcinogens including polycyclic aromatic hydrocarbons, are associated with numerous cancers including oral cancer, lung cancer, bladder cancer and colorectal cancer (see table 1). A polymorphism in *NAT* gene increases the risk of bladder cancer in individuals exposed to arylamines from occupational exposures, but has no effect without exposure (Ross *et al* 1996; Risch *et al* 1995; Hein *et al* 2000).

DNA repair enzymes have an important role in protecting the genome from the endogenous (reactive oxygen species) and exogenous (environmental carcinogens, UV light, *g* radiation etc.) exposures. Disruption of the function of DNA repair genes is associated with increased

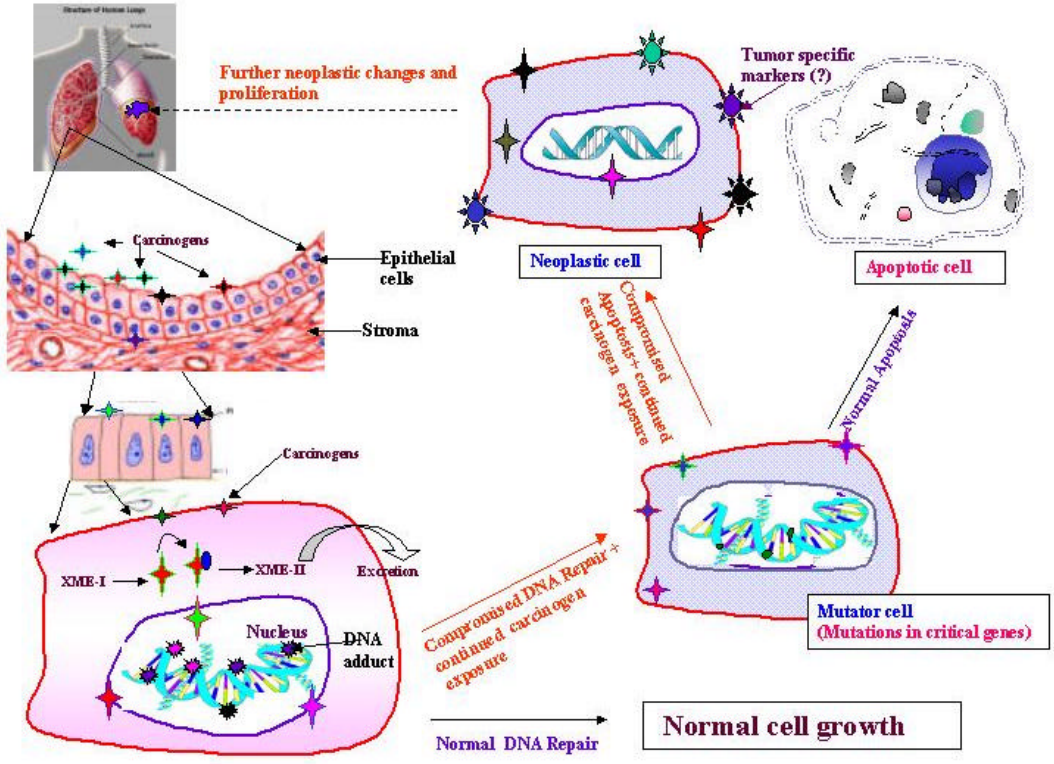

Figure 3. Genetic model for sporadic lung cancer.

sensitivity to DNA damaging agents and cancer proneness (Ishikawa *et al* 2001). Hence, genes encoding for the DNA repair molecules are candidate cancer susceptibility genes. One of the DNA repair genes, XRCC1 exon 10 variant genotype, has been shown to be associated with increased risk for head and neck cancer (Sturgis *et al* 1999) and gastric cancer (Shen *et al* 2000).

Polymorphisms in some of the gatekeeper genes are associated with cancer susceptibility. Several polymorphisms have been reported in the *p53* gene of which polymorphism at codon 72 has been implicated in modulating the response to environmental carcinogens by altering apoptotic response in the cell (Fan *et al* 2000). CHEK2 is another gatekeeper gene involved in cell cycle check point which codes for a kinase implicated in DNA repair. CHEK2 1100delC is a truncated variant which abrogates kinase activity and is associated with sporadic breast cancer (figure 2) (Meijers-Heijboer *et al* 2002).

A list of polymorphisms associated with some of the common cancers is given in table 1. A more exhaustive list of SNPs investigated in cancer case-control studies is given in Zhu *et al* (2004). In addition to these genes there are genes involved in immune system regulation which may be important in viral infections and haematopoietic cancers, as well as genes that regulate behaviour which may affect cancer risk (Brennan 2002).

Table 1. Polymorphisms in low penetrance genes associated with some of the common cancers.

Gene	Nucleotide/amino acid change	Associated cancer	Associated exposure	Mechanism of action	Reference
CYP1A1	3′non-coding region 6235 T > C	Breast, uterine	Oestrogen metabolites	Activating pro-carcinogens and catalyzing oxidative metabolites of oestrogen	Peto and Houlston 2001
CYP1A1	Codon 462 Exon 7 Ile–Val	Lung	Tobacco habit	Activation of tobacco related PAH	London *et al* 2000
CYP1A2	5347 T > C	Lung, bladder, colorectal	Tobacco habit	Activation of nitrosamines and arylamines	Seow *et al* 2001
GSTM1 *GSTT1*	Deletion (null genotype)	Lung, bladder, breast, HNSCC, colon, uterine, stomach	Tobacco habit	Carcinogen detoxification of oxidative metabolites	Miller *et al* 2002; Jhavar *et al* 2004
NAT2	C282T and T341C	Bladder, colon, liver	Tobacco habit	Carcinogen detoxification of aromatic amines, hydrazines	Hsieh *et al* 1999; Hein *et al* 2000; Tiemersma *et al* 2004
CHEK2	1100 del. C (truncating variant), missense variant I157T	Breast, prostate		DNA damage and replication checkpoint	Varley and Haber 2003; Cybulski *et al* 2004
p53	Codon 72 (Arg–Pro)	Lung	Tobacco habit	Apoptosis regulation	Fan *et al* 2000
XRCCI	Codon Arg399Gln Arg194Trp	Breast, oesophageal cancer, HNSCC	Tobacco habit	DNA repair	Shu *et al* 2003; Xing *et al* 2002
hOGGI	Ser326Cys	Lung	Exposure to tobacco smoke	Oxidatively damaged DNA repair e.g. 8-oxo-G DNA adducts	Park *et al* 2004
SULT1A1	Arg213His	Breast, bladder	Oestrogen, tobacco	Catalyzes the sulfation of phenolic and estrogenic compounds, metabolism of polycyclic aromatic hydrocarbons (PAHs) and aromatic amines	Han *et al* 2004
Alcohol dehydrogenase 3 (*ADH3*)	Ile349Val	UADT, colorectal adenomas	Alcohol	Alcohol metabolism	Nishimoto *et al* 2004

7. Epigenetic changes in low penetrance genes

Besides genetic polymorphisms, there could be non-structural changes in the genes such as epigenetic changes which influence protein expression or protein processing thereby altering the association between genotype and phenotype. Epigenetic changes are brought about by DNA methylation in which a family of DNA methyltransferase enzymes covalently modify cytosine by adding a methyl group in the CpG dinucleotides (Costello and Plass 2001). DNA methylation is thought to result in gene silencing, gene activation and chromosomal instability. One of the examples is the role of E-cadherin gene in epithelial cancers. In many cancers E-cadherin, which is a cell adhesion molecule, is silenced by DNA methylation of the gene (Chen *et al* 2004).

8. Low penetrance genes, carcinogen exposure and common cancers

About 10% of all breast cancers are familial, out of which only 20% are due to strong predisposing genes such as *BRCA1* and *BRCA2* (Balmain *et al* 2003). Mutations in *BRCA1* and *BRCA2* confer a high risk of breast and ovarian cancer. The remaining 90% of all breast cancers are due to unknown predisposing genes and their interaction with environmental factors. Until recently, no direct link between exposure to carcinogens, genetic alteration and human cancer could be drawn. Now there is some evidence that interindividual variability represented as genetic polymorphisms, associated with prolonged exposure to increased levels of oestrogen, may define a sub-set of women with breast cancer (Yager 2000; Sparks *et al* 2004; Tworoger *et al* 2004). Oxidative metabolites of oestrogen are known to cause DNA damage (Yager 2000). Polymorphisms in genes involved in oestrogen biosynthesis, and the conversion of oestrogen metabolites and their by products could be the low penetrance genes conferring risk in the etiology of sporadic breast cancers (figure 2) (Mitrunen and Hirvonen 2003; Thompson and Ambrosone 2000). Higher lifetime oestrogen exposure and inter-individual variability might identify women with increased genetic lesions in the breast tissue and therefore increased risk of breast cancer. In addition to endogenous factors, life-style factors such as smoking and alcohol use could contribute greatly to sporadic breast cancers.

Some of the most important known causes of cancer in the UADT are tobacco habit, obesity and oncogenic viruses. Supported by studies in cancer epidemiology, the carcinogenic effect of tobacco has been established beyond doubt, with the incidence of lung cancer increasing in individuals who start smoking early in life and continue smoking through out life (Peto 2001). The carcinogenic effect of tobacco is known to have a synergistic effect with alcohol (Day *et al* 1994). The importance of life-styles such as use of tobacco, alcohol as well as role of diet have been well studied for head and neck squamous cell carcinoma (HNSCC). However, host susceptibility must play an equally important role since only a small percentage of individuals with these habits actually develop cancer. Further, about 20% of patients with tobacco-related malignancies develop a second primary cancer (Warnakulasuriya *et al* 2003). This forms an important sub-set of patients in whom genetic predisposition may be over-represented and can be compared to patients with single primary HNSCC or control, healthy individuals with similar exposures. Cumulative effect of polymorphisms in caretaker genes as well as gatekeeper genes involved in pro-carcinogen metabolism of tobacco-related polycyclic aromatic hydrocarbons would increase risk of HNSCC (figure 3).

9. Future of low penetrance genes

With the sequencing of the human genome and the technological advances, it is now possible to carry out high throughput genotyping of polymorphisms in several low penetrance candidate genes in large epidemiological studies. So far, due to small sample number of cases or selection of a small subset of low penetrance genes, the evidence that they are cancer-susceptibility genes is statistically weak and often conflicting. In the two large scale studies published, no association between polymorphisms in the XME genes studied and tobacco consumption was found (Smits *et al* 2004), or the analysis supported modest associations of *GSTM1* and *GSTT1* genotypes with head and neck cancer risk (Hashibe *et al* 2003). We are of the opinion that the cumulative effect of polymorphisms in the low penetrance genes predisposes individuals to cancer. Hence, polymorphisms in selected (based on the exposure) XMEs, DNA repair genes as well as gatekeeper genes have to be studied to identify the individuals at high risk.

In order to get statistically significant results in a smaller number of patients, some SNP prioritization can be done by enriching patient categories by susceptible individuals such as familial, early onset or patients with multiple primary cancers (Imyanitov *et al* 2004). Identification of low penetrance genes involved in genetic susceptibility is important especially in cancers which are known to be associated with lifestyles such as diet, tobacco and alcohol. Better understanding of the role of various low penetrance genes and various gene-gene and gene-environment interactions would enable us to understand the genesis of the common sporadic cancers, identify high risk individuals for cancer development and devise appropriate preventive/ screening strategies for them.

10. Conclusions

With the advances in technology, the complete genotyping of every individual may soon become possible. However, the importance of genes has been overstated by the media to the lay public. Genetic determinism is a notion that we are what we are because of our genes. This is true to a large extent although genes do not function in isolation. They respond to signals received from the external milieu – either from cell surface molecules on adjacent normal cells or from chemicals (hormones, growth factors, carcinogens) in the microenvironment or due to epigenetic changes. The public perception of genetic determinism is that if we know the genetic make-up of an individual, we can predict what befalls him/her. Many believe that a clone of a great star will have the same attributes of the star. The clone may not be identical as it will have grown in a different microenvironment with different external stimuli and the genes turned *on* or *off* could be different. Apart from the social implications, genetic determinism is implied in disease states as well. However in case of polygenic disorders like cancer, diabetes, obesity etc. it is the genes in combination with the environment, which determine the susceptibility to the disease. Nurture and nature together determine the individual's fate.

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