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Effect of Genetic Polymorphisms on Urinary Bladder Neoplasms

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To my family

ABSTRACT

Urinary bladder neoplasms (UBNs) are among the most common malignancies in the world and are mainly attributed to exogenous risk factors such as, smoking, exposure to industrial aromatic amines and certain medicinal drugs which cause DNA damage. The entire process leading to DNA damage and subsequent repair of the damage involves a plethora of enzymes including those involved in metabolic and DNA repair processes. Genetic polymorphisms in the genes encoding enzymes involved in metabolism and DNA repair can potentially modulate susceptibility to cancer as well as alter the clinical course and treatment response in this disease. Despite previous studies, a comprehensive investigation on the role of polymorphisms in the genes involved in metabolism and DNA repair in UBNs has been lacking.

The main objective of this thesis was to investigate the role of polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes in the susceptibility, pathogenesis and clinical behavior of UBNs. The cases included bladder cancer patients, diagnosed during 1995-96 in different hospitals of the Stockholm County area. Follow-up information about the cases was recorded for 5 years. The controls were also drawn from the Swedish population. Both the cases and controls were genotyped for polymorphisms in different DNA repair, cell-cycle regulatory and metabolic genes.

The results from the population based association studies (paper I & II) showed that the variant allele homozygotes of the A499V (C>T) and K939Q (A>C) polymorphisms in the XPC gene were associated with 6- and 3-fold increased risk of UBNs, respectively. Moreover, the variant allele genotypes of the A499V (C>T) polymorphism were associated with decreased age of disease onset. Increased risk of disease was also observed with the variant allele haplotypes of these two polymorphisms. Increasing number of XPC variant alleles (499V and 939Q) in the genotypes increased the risk of UBNs in a linear dose dependent manner. We also observed a 2.5 times higher risk of UBNs in the individuals lacking the GSTT1 gene than those who did not. In contrast, the variant allele homozygote genotype of H27H (T>C) polymorphism in the H-ras gene was associated with a decreased risk of UBNs.

Our studies on the influence of polymorphisms on the disease course and clinical outcomes in UBNs (paper III & IV) showed that patients, who were simultaneous carriers of variant alleles for the K751Q (A>C) and K939Q (A>C) polymorphisms in the XPD and XPC genes, respectively had longer disease free survival than the rest of the patients. Longer disease free survival was also observed in the carriers than the non-carriers of variant allele for A222V (C>T) polymorphism in the MTHFR gene. In contrast, compared to the non-carriers, carriers of the variant allele for R139W (C>T) polymorphism in the NQO1 gene showed shorter disease free survival in T1 disease. The variant allele carriers of R399Q (G>A) polymorphism in the XRCC1 gene were at reduced risk for recurrence and death after instillation and radiotherapy. A significant low-risk for stage progression was observed in patients carrying the variant allele for H27H (T>C) polymorphism in the H-ras gene. The variant allele carriers of G39E (G>A), P187S (C>T) and I105V (A>G) polymorphisms in the MSH6, NQO1 and GSTP1 genes, respectively showed association with increased risk for highly malignant UBNs.

In conclusion, our results are consistent with the notion that the DNA repair, cell-cycle regulatory and metabolic gene polymorphisms influence the susceptibility to UBNs as well as the disease course and outcomes in patients with this disease.

CONTENTS

I. LIST OF PUBLICATIONS

This thesis is based on the following articles that will be referred to by their Roman numerals.

I. **Sanyal S**, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkstorm H, Larsson P, Kumar R, Hemminki K. Polymorphisms in DNA repair and metabolic genes in bladder cancer. *Carcinogenesis. 2004 May; 25(5):729-34*.

II. **Sanyal S**, de Verdier PJ, Bermejo JL, Steineck G, Hemminki K, Kumar R. Association between polymorphisms in the XPC gene and risk of urinary bladder neoplasms. *Submitted*.

III. **Sanyal S**, de Verdier PJ, Steineck G, Larsson P, Onelöv E, Hemminki K, Kumar R. Polymorphisms in *XPD*, *XPC* and the risk of death in patients with urinary bladder neoplasms. *Acta Oncologica, 2006 (In press)*

IV. **Sanyal S**, Ryk C, de Verdier PJ, Steineck G, Larsson P, Onelöv E, Hemminki K, Kumar R. Polymorphisms in NQO1 and the clinical course of urinary bladder neoplasms. *Scandinavian journal of urology and nephrology, 2006(In press)*

II. LIST OF ABBREVIATIONS

APEX1	Apurinic/apyrimidinic endonuclease-1
ARF	Alternative reading frame
ATM kinase	Ataxia telangiectasia mutated kinase
BCG	Bacillus calmette guerin
BER	Base excision repair
BRCA1	Breast cancer associated protein 1
CA	Chromosomal aberration
CCNH	Kinase subunit cyclin H
CDK	Cyclin-dependent kinase
CDKN	Cyclin dependent kinase inhibitor
CIS	Carcinoma *in situ*
CS	Cockayne syndrome
DBC1	Deleted in bladder cancer 1
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DSB	Double strand break
Elk1	ETS like transcription factor 1
ERCC1	Excision repair cross complementing 1
ERK	Extra-cellular signal-regulated kinase
FEN1	Flap-endonuclease 1
FGFR3	Fibroblast growth factor receptor 3
GAP	GTPase-activating proteins
GDP	Guanosine di phosphate
GRB2	Growth factor receptor-bound protein 2
GST	Glutathione S-transferases
GTP	Guanosine tri phosphate
HR23B	homolog Rad23B
INK4a	Inhibitor of cyclin-dependent kinase 4
LD	Linkage disequilibrium
LOH	Loss of heterozygosity
MAPK	Mitogen activated protein kinase
MDM2	Murine double minute 2
MEK	MAPK/ERK kinase
MLH	MutL protein homolog
MMC	Mitomycine C
MMR	Mismatch repair
MSH	MutS homolog
MTHFR	Methylene-tetrahydrofolate reductase
NAT2	N-acetyltransferase 2
NBS1	Nijmegen breakage syndrome 1
NER	Nucleotide excision repair
NERC	Nucleotide excision repair capacity
NQO1	NAD(P)H dehydrogenase quinine 1
OGG1	8-Oxoguanine DNA glycosylase
PARP1	poly-adinosine diphosphate-ribose polymerase

PCNA	Proliferating cell nuclear antigen
PCR-RFLP	Polymerase chain reaction-Restriction fragment length polymorphism
PMS2	Post-meiotic segregation increased 2
PRAD1	Parathyroid adenomatosis 1
PTCH	Patched homolog
PTEN	Phosphatase and tensin homolog
RB1	Retinoblastoma 1
RFC	Replication factor C
RPA	Replication protein A
RTK	Receptor tyrosine kinase
SCC	Squamous cell carcinoma
SNP	Single nucleotide polymorphism
SOS	Son of sevenless homolog 1
SSB	Single strand break
TFIIH	Transcription factor II H
TSC1	Tuberous sclerosis 1
TURB	Transurethral resection of bladder
UBNs	Urinary bladder neoplasms
UCC	Urothelial cell carcinoma
WHO	World health organization
XP	Xeroderma pigmentosum
XRCC	X-ray repair cross-complementing

1. INTRODUCTION

Cancer accounts for more than 20 percent of all deaths in the world each year and is one of the most important medico-biological problems of this century. The central event in cancer development is loss of genomic integrity which itself probably initiates from the assortment of genomic DNA by exogenous or endogenous carcinogens [1].

Like several other cancers, exposure to exogenous carcinogens is one of the causal factors for urinary bladder neoplasms (UBNs). The risk factors for UBNs include smoking, exposure to industrial aromatic amines and some medicinal drugs such as phenacetin [2]. Exposure to such exogenous agents and by-products of cellular metabolism results in DNA damage which, left un-repaired, can lead to carcinogenesis through mutation fixation and clonal expansion of cells carrying mutations in the critical genes. The entire process leading to DNA damage and subsequent repair of the damage involves different enzymes, including those involved in metabolic and DNA repair processes. Therefore, functional optimality in the metabolic and/or DNA repair processes is the pre-requisite of reduced cancer risk while the risk increases with the sub-optimality in these processes. The capacity to metabolize carcinogens or to repair DNA damage under uniform exposure varies between the individuals. The inter-individual variability in these processes is mostly attributed to the genetic polymorphisms in the genes involved in metabolism and DNA repair. Polymorphisms may result in subtle structural alterations of metabolic and repair enzymes and can have an impact on metabolic and DNA repair processes. The genetic polymorphisms are considered as the main modulators of individual repair and metabolic capacities and consequently risk of cancer. Polymorphisms in the genes encoding enzymes involved in metabolism and DNA repair can also potentially influence the clinical course and outcomes in the disease [3-6]. The risk of UBNs as well as their clinical courses are reported to be modulated by the genetic polymorphisms in genes encoding either metabolic, DNA repair or cell-cycle regulatory enzymes [7-14]. Despite previous studies, a comprehensive investigation of such genetic risk factors in UBNs has been lacking.

One objective of this thesis was to study the role of polymorphisms in genes involved in DNA repair, metabolism and cell-cycle regulation and susceptibility to UBNs. The other objective was to explore the influence of investigated polymorphisms on disease course and clinical outcome in patients with the disease.

2. BACKGROUND

2.1 Urinary bladder neoplasms

2.1.1 Incidence

Urothelium, the inner lining of entire urinary tract, act as a permeable barrier between the urine and blood and is continuously exposed to a variety of potential carcinogens that are generally excreted by the kidneys and the urinary tract. Therefore, it is of no surprise that urinary bladder neoplasms are among the most common malignancies in the world [15]. However, the incidence rate of UBNs varies significantly throughout the world (≈ 10 folds). Countries with particularly high incidence rate of urothelial cell carcinoma (UCC) of the bladder include Italy, Spain, Denmark, UK and US. Countries with low incidence rate of UCC are Japan and China [2]. Unlike UCC, incidence of squamous cell carcinoma (SCC) of the bladder is rare in the western world while, it is the most common malignancy in the Nile river valley where the parasite *Schistosoma haematobium* is endemic. The incidence increases with age and peaks at 80 years of age or older. The age adjusted incidence in Sweden is approximately 2000 new cases per year with a five year cancer specific survival of 71 percent. This is the fourth most common cancer in men and the ninth most common cancer in women with a male to female ratio of 3:1 [16].

2.1.2 Classification

Bladder tumors are generally classified on the basis of their histology, patterns of growth (e.g. papillary vs. non papillary, invasive vs. noninvasive) and grade of malignancy. The most common histological type is UCC that constitutes 90 percent of all primary bladder tumors. Where as, only 10 percent of primary bladder tumors are represented by squamous cell carcinoma, adenocarcinoma and small cell carcinoma. According to the growth pattern, bladder tumors are classified into different stages with the TNM system, which includes information about primary tumor (T), nodal involvement (N) and metastasis (M) [17]. Primary tumors (T) are classified as non invasive papillary carcinomas (Ta), infiltrative carcinomas that extend into lamina propria and invade the sub epithelial connective tissue (T1), the muscular layer (T2+), or as superficial flat carcinoma *in situ* (CIS) (Fig. 1a). Depending on the cytological grade of malignancy bladder tumors are subdivided into five different malignant grades namely, G1 (well differentiated, Fig 1d), G2 (moderately differentiated), G3 (poorly differentiated), G4 (undifferentiated, Fig 1e) and Gx (grade cannot be assessed) [18, 19]. Malmstrom et al further subdivided the G2 into G2a (more like G1) and G2b

(more like G3) [19]. Based on the clinical outcomes of patients Larsson et al have classified the disease into five different disease categories (TIS, TaG1, TaG2, TaG3+T1 and T2+) [20]. According to the 2004 WHO classification of bladder tumors, papillary urothelial neoplasm of low malignant potential (TaG1) no longer carries the label of 'cancer' [21].

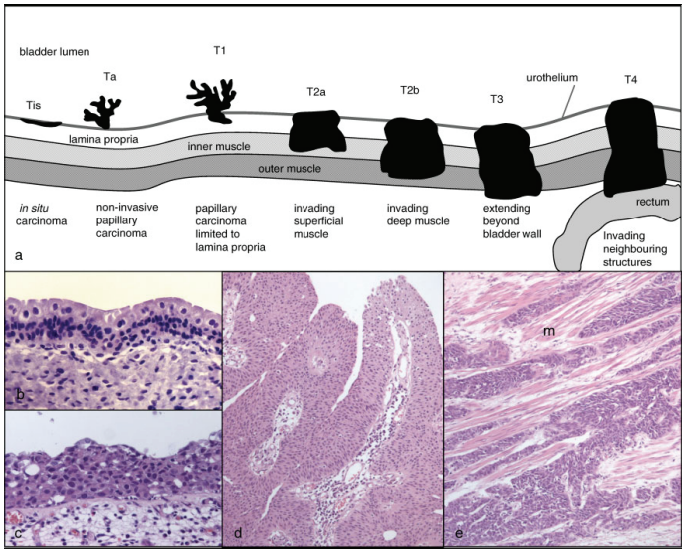

Figure 1. ***(a)*** *Different stages of urinary bladder tumors. Stage T2a, T2b, T3 and T4 represent T2+.* ***(b–e)*** *Haematoxylin and eosin stained sections.* ***(b)*** *Normal urothelium with the large differentiated superficial cells.* ***(c)*** *CIS. Cells are disorganized and show marked nuclear atypia (high-grade).* ***(d)*** *Low-grade superficial papillary tumor.* ***(e)*** *High-grade tumor invading muscle (m) [Reprinted by permission from Carcinogenesis [22] copyright (2006) Oxford University Press].*

2.1.3 Prognosis

More than 70 percent of bladder tumors are non-invasive (Ta/T1) at diagnosis. Low grade papillary Ta tumors (i.e. TaG1) almost never progress and patients with this disease seldom die due to the disease (Fig 2). Though, a 50-70 percent recurrence for TaG2 tumors is reported, yet those are associated with good prognosis. A majority of patients with T1 disease also show good prognosis in response to Bacillus Calmette-Guerin (BCG) treatment. However, about one third of the patients do not respond to BCG. In such cases the tumor progresses to invade the muscle and outcome is death due to the disease (Fig.2) [20]. *Carcinoma in situ* (CIS ~ 10 percent) is a flat, high grade tumor that initially remains within mucosal boundaries (Fig 1a & c) but is believed to be a precursor of invasive bladder cancer as it is often found together with

invasive UCC [23]. Sixty percent of patients with this type of tumor have a poor prognosis [24]. In contrast to the superficial papillary tumors, the tumors that are muscle-invasive at diagnosis (T2+; ≈ 20 percent) have a poor prognosis (Fig.2). Approximately 50 percent of these patients develop lethal distant metastasis and die due to the disease in spite of radical surgery, radiation or chemotherapy [23].

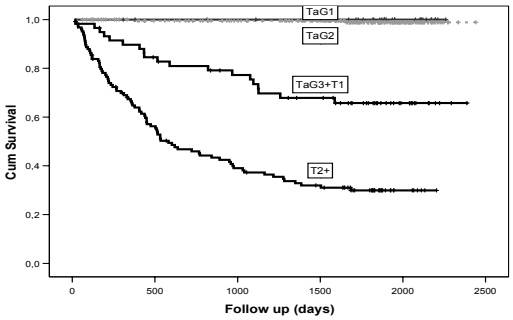

Figure 2. *Disease specific survival in patients with different disease categories in UBNs*

2.1.4 Treatment

The choice of treatment in urinary bladder neoplasms depends on different factors including tumor size, stage, grade, involvement of lymph nodes, presence of metastasis, and physical condition of the patients. Low grade tumors without muscle invasion are mainly treated by transurethral resection (TURB). In case of multiple or recurrent low-grade tumors TURB is supplemented with intravesical chemotherapy (e.g. Farmorubicine, Doxorubicine, and Mitomycine C) or radiation therapy [25-28]. Intravescical immunotherapy with BCG is also used for the treatment of patients with recurrent non-invasive tumors and has been shown to give prolonged protection from tumor recurrence in about two thirds of the patients [29]. High grade muscle-invasive bladder tumors are best treated by radical cystectomy provided that no metastasis is present at diagnosis. Chemotherapy in a neo-adjuvant setting is mainly used to decrease the size in a locally advanced bladder tumor followed by cystectomy, while adjuvant chemotherapy is given in node positive cases [30]. Radiation therapy allows organ preservation and is only given to selected patients with muscle-invasive bladder tumors [31].

2.1.5 Etiology / Risk factors

2.1.5.a Environmental risk factors

The environmental risk factors of UBNs are well investigated and established. The most common known etiologic factor of UCC is cigarette smoking. In addition, numerous specific chemicals have been identified as bladder carcinogens. These include aromatic amines and amides (e.g. 4-aminobiphenyl, benzidine and 2-naphthylamine), phenacetin-containing analgesics, and certain cancer chemotherapeutic agents (e.g. phosphoramide mustards). Occupational exposure to various combustion gases including those found in aluminum industries and in diesel exhaust has been found to increase the risk of UBNs. Besides, exposure to arsenic in drinking water probably also increases the risk of bladder neoplasia [32]. Various heterocyclic amines generated by pyrolysis of foods especially red meat, have been suggested as potential dietary risk factors for UBNs [2]. Unlike UCC, squamous cell carcinoma (SCC) is rare in western world but is common in Egypt and Middle East where the parasite *Schistosoma haematobium* is endemic. This parasite causes Scistosomiasis, a chronic inflammation, which is believed to cause SCC [33].

2.1.5.b Genetic risk factors and the molecular alteration in bladder tissues

Although molecular alterations are common in bladder tumor tissues and even though subjects with a family history of UBNs have a slightly increased risk of this disease, no disease causing gene has been identified [34]. Somatic genetic alterations observed in bladder tumor tissues include activation of proto-oncogenes (FGFR3, H-ras, Cyclin D1 and MDM2) and inactivation of tumor suppressor genes (p53, CDKN2A/ARF, RB1 and PTEN genes). Mutations, non-random deletion or gain at multiple chromosomal sites and epigenetic alterations are cause of genetic heterogeneity of bladder tumors. The most frequent losses are found on chromosome 9. Candidate genes in this chromosome are CDKN2A/ARF, CDKN2B, PTCH, DBC1 and TSC1. Losses are also common in chromosomes 11p, 17p, 8p, 4p, 13q, 3p, 10q and 18q. In contrast to the losses, gains of 1q, 5p, 8q and 17q have also been found. Despite genetic heterogeneity, somatic alterations in bladder cancer display specific patterns in different disease categories. The activating mutations of the FGFR3 gene are common in low grade non-invasive bladder tumors while, inactivating mutations in p53 gene are often observed in high grade invasive tumors (Fig 3) [22, 35, 36].

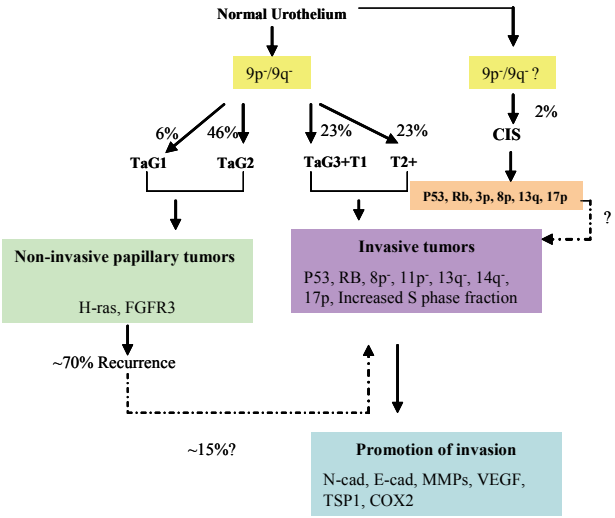

Figure 3. *Model for bladder cancer progression showing the molecular pathways of tumorigenesis. Deletion of both arms of chromosome 9 ($9p^-/9q^-$) occurs early during urothelial tumorigenesis Non-invasive papillary tumors (TaG1 and TaG2) harbor frequent mutations in the H-ras (30–40%) and fibroblast growth factor receptor 3 (FGFR3) gene (70%). High-grade muscle-invasive tumors contain structural and functional defects in the tumor suppressors' p53 and/or the retinoblastoma protein (RB). An increased S phase fraction has also been observed in this kind of tumors. Invasion and metastases are promoted by several factors that alter the tumor microenvironment, including the aberrant expression of N- and E-cadherins (N-cad and E-cad), matrix metalloproteinases (MMPs), angiogenic factors such as vascular endothelial growth factor (VEGF), and antiangiogenic factors such as thrombospondin 1 (TSP1) and cyclooxygenase 2 (COX2).*

2.1.6 Susceptibility

Not every individual with an exposure to risk factors develop urinary bladder neoplasms. At the same time, many patients develop the disease without being exposed to any of the known risk factors [2]. Such discrepancies in disease development arise from the genetically predetermined inter-individual variation in susceptibility. The susceptibility may be low or high; it is polygenic and follows a normal distribution in the population. The genetic characteristics that determine individual's susceptibility to cancer is modulated by the "**genetic polymorphisms"**; germline variations in DNA sequences. The mechanisms by which genetic polymorphisms are postulated to modulate the susceptibility of UBNs are discussed in the subsequent chapters.

2.2 Genetic polymorphisms

Genetic polymorphisms are defined as germline variations in DNA sequences that result to produce alternative forms of one gene which co-exist in more than one percent of the population. Alternative forms of a polymorphic gene that exists at single locus are known as “alleles”. A common or wild type allele is represented by most of the individuals in a population. The other allele, which is represented by fewer individuals in the population, is called a rare or variant allele. However, the frequency of a certain allele varies typically among different populations, and thus the classification of an allele as ‘rare’ or ‘common’ is population specific.

Polymorphisms can either result from substitution of one base for another or from the insertion or deletion of a section of DNA and also from gross genetic losses and rearrangements. The simplest type of all polymorphisms is the **single nucleotide polymorphism (SNP),** which accounts for 90 percent of all genetic polymorphisms and can occur as frequently as 1 per 300 base pairs [37, 38]. There are probably >10 million SNPs in the human population. SNPs within the coding region can alter protein structure (non-synonymous) and some intronic SNPs can affect splicing of a gene. SNPs within the 3’-untranslated region can alter mRNA stability or influence transcription if present in the promoter region of a gene. Though, in the majority of cases polymorphism within introns or inter-genic regions cause no discernable effect [39, 40].

2.2.1 Genetic polymorphisms and cancer susceptibility

Genes involved in carcinogen metabolism, DNA repair and cell cycle regulation co-ordinate to facilitate the initial defenses against cancer development. Metabolic enzymes detoxify carcinogens to provide the first line of defense. The enzymes encoded by DNA repair genes counteract the consequences of mutagenic exposure of cells and prevent carcinogenesis by removal of damaged DNA bases [41]. In parallel, the products of cell-cycle regulatory genes ensure error-free replication of DNA by regulating cell-cycle progression with a provision for DNA repair (Fig. 4) [42]. Polymorphisms that cause subtle structural/functional alteration of metabolic, DNA repair and cell-cycle regulatory proteins can influence these processes, hence individual susceptibility to carcinogenesis and therefore are postulated to serve as ‘susceptibility markers’ of the disease.

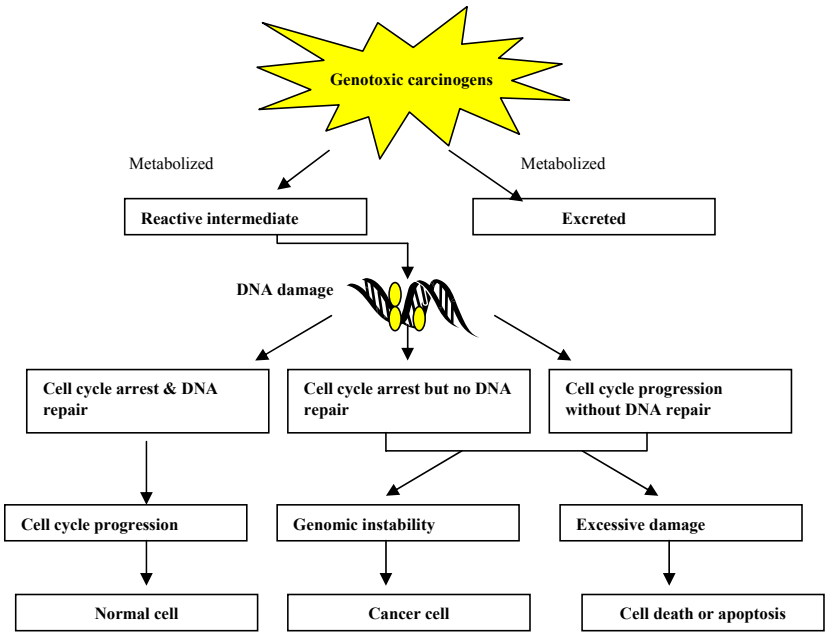

Figure 4. *Co-ordination between the metabolic, DNA repair and cell-cycle regulatory processes. Reactive metabolites cause DNA damage. Before progression of cell-cycle, the repair of damage is necessary to maintain the genomic integrity. Un-repaired DNA lesions can lead to transformation of cells through mutation fixation and clonal expansion of damaged cells. Excessive damage can also lead to cell death or apoptosis, which is an anti-carcinogenesis process.*

2.2.2 Genetic polymorphisms, progression and outcomes in cancer

Besides modulating the susceptibility, inter-individual genetic variation can also contribute to the prognosis and outcomes of cancer. Candidate genes that influence tumor progression are oncogenes, cell-cycle regulatory genes, and also genes involved in the immune response, inflammatory change, neovascularization, and extra-cellular matrix breakdown. Metabolic and DNA repair genes can also influence tumor progression and disease outcomes [43]. Polymorphisms in DNA repair and metabolic genes may also influence the occurrence and type of somatic mutations and chromosomal alterations and can thus predict the pathogenesis or progression of the tumor [44]. For example in urinary bladder tumors p53 mutations, which are associated with invasive tumors, are reported to be influenced by the polymorphisms in DNA repair and metabolic genes [45-48].

Polymorphisms in DNA repair and metabolic genes can also be potentially used in pharmcogenetics, which aims to identify individuals predisposed to high risk of toxicity from conventional doses of cancer chemotherapeutic agents [49].

Polymorphisms in the drug metabolic genes could influence the efficacy of the chemotherapeutic drugs by affecting their activation. Polymorphisms in the DNA repair genes can also influence the response to chemo and radiotherapy. These therapies are based on the premise of selective killing of cancer cells through DNA damage. Therefore altered repair capacities can result in different outcomes. A number of studies have reported associations between polymorphic variants of metabolic and DNA repair genes and outcomes of treatments with chemo or radiotherapy [8, 12, 50-52].

2.2.3 Degrees of association between genetic polymorphisms and cancer

The variant alleles of single nucleotide polymorphisms are usually associated with low penetrance and the effect on familial risk of cancer is generally moderate. The impact on susceptibility of variant alleles becomes significant when the risk is estimated at the population level with a large sample size [53]. Due to low penetrance pathogenic alleles per se are not sufficient to develop a particular phenotype and require additional genetic events [54]. The effect of a specific polymorphic allele to the risk of disease could also be context dependent and show opposite effect between two different populations [55].The effect usually manifests due to combined effect of a collection of polymorphic alleles in sets of key genes, and environmental factors that determine together whether an individual will suffer from a disease or not [39].

Because of usually low penetrance of a single pathogenic allele, it is imperative to study other polymorphic alleles which are in linkage disequilibrium [39]. Linkage disequilibrium (LD) is the non-random association of alleles at different genetic loci. Alleles that are placed close together on a chromosome show high LD and tend to inherit together. A set of such alleles on a chromosome or a part of chromosome is called **haplotype**. Different haplotypes with combinations of particular alleles in the same gene could influence the function of the gene and thus its penetrance [56]. Moreover, unlike a single SNP marker haplotypes help to demonstrate the association of a region of the genome with the disease. Haplotypes within a gene can also reflect historical linkage to other functional loci or genes [57].

2.3 Polymorphisms of carcinogen metabolic genes and the risk of cancer

2.3.1 The carcinogen metabolic systems

At an early stage, most types of cancers are initiated by carcinogens or xenobiotics which can be both of exogenous and endogenous origin. Tobacco smoke, food, and environmental as well as work place pollutants are the sources of exogenous carcinogens. UV component of light and radioactive irradiations are also among the known carcinogens. Most of these exogenous agents require metabolic activation prior to reaction with macromolecules like DNA and protein.

The main function of the carcinogen metabolic system is to bio-transform the lipophilic pre-carcinogens into readily excretal hydrophilic metabolites. The enzymes involved in carcinogen metabolism, despite certain overlaps, are traditionally classified as phase I and phase II enzymes [58]. The **phase I** detoxification system, composed of the cytochrome P450 super-gene family of enzymes, is generally the first enzymatic defense against foreign compounds. The phase I enzymes besides increasing the water solubility of the carcinogens make those more reactive through reduction, oxidation or hydrolysis reactions [59, 60]. The metabolites from phase I reactions undergo detoxification by the **phase II** enzymes. This group of enzymes conjugate ionized groups (e.g. glutathione, methyl or acetyl) to the activated metabolites, converting those into less reactive, more water soluble and excretal (Fig. 5) [61]. Recently, antiporter activity has been defined as a **phase III** system which by pumping out reduces the intracellular concentration of xenobiotics in the cells. The carcinogen metabolic enzymes also play important roles in drug metabolism [62].

Polymorphisms in phase I and II metabolic genes influence the induction as well as function of metabolic enzymes and hence alter individual carcinogen and drug metabolic capacity [6, 61, 63, 64]. However, the impact of polymorphisms on the efficacy of enzymes depends on the metabolic phase the enzyme is involved in and also on the effectiveness of the other phase [65]. For example, a rapid phase I in combination with a slow phase II metabolism may result in accumulation of toxic metabolites which are potent carcinogens and react with macromolecules, like DNA and RNA. While, a slow phase I with effective phase II metabolism ensures proper detoxification and excretion of the metabolites from the body and thus protection of genomic DNA. However, a slow phase I metabolism may also lead to toxic damage if

the parental substance is more toxic than its metabolites. Therefore, a proper balance between the phase I and phase II systems is necessary to avoid carcinogenicity and violation of that can dramatically alter xenobiotic toxicity or therapeutic efficacy [65].

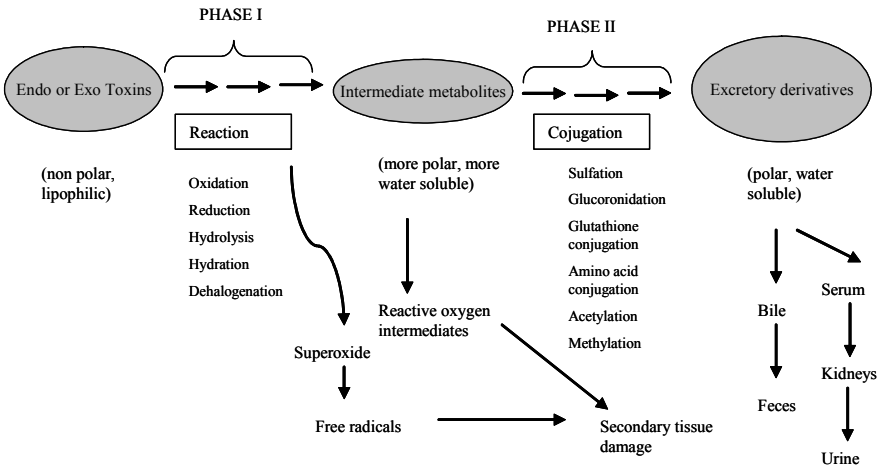

Figure 5. *Summary of detoxification pathways for phase I and phase II reactions*.

2.3.2 Risk of cancer with different metabolic gene polymorphisms

Genetic polymorphisms in several phase I and phase II metabolic enzymes are associated with the risk of UBNs. These includes polymorphisms in the NQO1, NAT2, GSTP1, GSTT1 and GSTM1 genes [66-68]. In addition polymorphisms in the folate metabolic gene, MTHFR, have also been associated with risk modulation of UBNs in some studies (Table 1).

NAD(P)H:quinone oxidoreductase 1 (NQO1), formerly known as diphtheria toxin diaphorase (DT-diaphorase), is located on chromosome 16q22. NQO1 is a cytosolic flavoenzyme that catalyzes the two-electron reduction of quinoid compounds into hydroquinones and protects cells from oxidative damages [69]. *In-vitro* evidence suggests that NQO1 activity can specifically reduce the rate of benzo(a)pyrene quinone-DNA adducts formation; generated by cytochrome P450 reductase [70]. Thus, NQO1 has been described as an anticancer enzyme. However, NQO1 also catalyzes some phase I reactions and bio-activates certain environmental pro-carcinogens, such as nitro-aromatic compounds and heterocyclic amines, present in tobacco smoke and certain processed foods [71, 72]. In addition to its dual function of detoxification and bio-activation of xenobiotics, NQO1 has also attracted wide attention as a drug-metabolizing enzyme for anti-tumor therapy. Many cytotoxic agents containing the

quinone moiety, such as the benzoquinone group, are activated by NQO1 via their reduction into hydroquinones [73]. Two nonsynonymous SNPs in NQO1 that have been discovered to date are: P187S (C>T) and R139W (C>T). Compared to the 187Proline allele the 187Serine allele of P187S polymorphism has shown lower enzymatic activity and been reported to influence the risk of lung, colorectal and urothelial carcinomas [63, 64, 74-77]. The R139W (C>T) polymorphism, although not well studied, has been found to reduce the enzymatic activity of NQO1 protein and increase the risk of infant acute lymphoblastic leukemia (ALL) [78, 79].

Table1. Summary of the effect of metabolic gene polymorphisms on the function of metabolic proteins and the risk of UBNs.

Genes (phase)	**Common variant / rare variant (codon)**	**Frequency of rare variant in Caucasian**	**Functional alteration with the variant allele**	**Ref. with respect to altered risk of UBNs**
NQO1 (I & II)	R/W (139)	0.03	Reduced activity	Increased risk [80-82]
	P/S (187)	0.18	Reduced activity with rapid degradation	
NAT2 (II)	Rapid/slow†	<0.50	Reduced expression/activity	Increased risk [83-86]
GSTP1 (II)	I/V (105)	0.30	Change in affinity/activity	Increased risk [87-90]
GSTT1 (II)	Non null/null	0.30	Absence of function	Increased risk [89, 91-95]
GSTM1 (II)	Non null/null	0.80	Absence of function	Increased risk [83, 91, 93, 96]
MTHFR (*)	A/V (222)	0.37	Reduced activity	Decreased risk [80]
	E/A(429)	0.37	Reduced activity	Increased risk [97]

(I) Involved in phase I metabolic reactions; (II) Involved in phase II metabolic reaction; (*) Involved in folate metabolism. † Slow variants of NAT2 are represented by NAT2*5, NAT2*6 and NAT2*7 alleles with respective frequencies of 0.46, 0.29, 0.03.in Caucasian.

Two distinct ***N*-acetyl transferases (NATs)**, NAT1 and NAT2, have been identified as being involved in the metabolism of aromatic amines and heterocyclic amines via *N*-, *O*-, or *N*, *O*-acetylation [98]. These enzymes are also involved in the acetylation of a number of aromatic amine and hydrazine drugs. Expression of NAT2 has been observed in liver where it detoxifies activated arylamines by *N*-acetylation. The NAT2 gene is highly polymorphic and is situated on chromosome 8p22. Several of the NAT2 alleles have been shown to change its enzymatic activity and are referred to

as slow or rapid acetylators, depending on their functional impacts. The NAT2 wild-type allele (NAT2*4) and four major mutant alleles – M1 (NAT2*5), M2 (NAT2*6), M3 (NAT2*7) and M4 (NAT2*14) have been identified [99]. The presence of two NAT2 mutant alleles produces a slow acetylation phenotype, whereas the presence of at least one wild-type allele produces a rapid or medium acetylator phenotype [99, 100]. Studies have reported association of NAT2 slow acetylators with the risk of bladder and prostate cancer, however such data are not conclusive [84, 85, 101, 102].

The **glutathione S-transferases (GSTs)** are a family of proteins which conjugate electrophilic molecules with glutathione to render them less toxic. Carcinogens detoxified by GSTs include polycyclic aromatic hydrocarbons present in the diet and tobacco smoke. Besides, a variety of anticancer drugs are also metabolized by the members of this group [103, 104]. Thus, genetic polymorphisms in these enzymes have high relevance for cancer susceptibility as well as for response to therapy [58]. Human GSTs have been grouped into 7 main classes: alpha, mu, omega, pi, sigma, theta and zeta [105].

The **glutathione S-transferases P1 (GSTP1)** gene belongs to the pi class, is present on chromosome 11q13 and shows 5 non synonymous SNPs. The I105V (A>G) polymorphism in the GSTP1 gene, affects the kinetic property of the enzyme. The 105Valine allele has been reported to be associated with a substantial reduction in the catalytic activity of the enzyme and the risk of several cancers including UBNs [106-108]. However, a contradictory protective effect of the 105Valine allele against cancer development has also been documented [108, 109].

Glutathione S-transferases M1 (GSTM1; class mu) and **glutathione S-transferases T1 (GSTT1**; class theta), show polymorphisms, due to large deletions in the structural genes. Homozygous deletions of these genes, result in null-genotypes and complete absence of the enzyme activities [103]. Null genotypes of GSTT1 and GSTM1 are present in a high percentage of the human population, with major ethnic differences. GSTT1 plays a major role in phase-II biotransformation of a number of drugs and industrial chemicals, (e.g. cyto-static drugs, hydrocarbons and halogenated hydrocarbons). The null genotype of GSTT1 has been reported to increase the risk for many malignancies including bladder neoplasms. GSTM1 is particularly relevant in the deactivation of carcinogenic intermediates of polycyclic aromatic hydrocarbons. Absence of this enzyme has been shown to be associated with lung, colon, colorectal and bladder cancer [103, 109-112].

Folate, the water soluble vitamin-B, modulates DNA methylation and is also important for the synthesis, stability and repair of DNA. The key regulatory enzyme involved in folate metabolism is **methylenetetrahydrofolate reductase (MTHFR)** that irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The 5,10-methylenetetrahydrofolate, is required for DNA synthesis [113, 114]. On the other hand, the 5-methyltetrahydrofolate is the precursor of methionine and S-adenosylmethionine (SAM) which are required for DNA methylation and protein synthesis. Two common non-synonymous SNPs, A222V (C>T) and E429A (A>C) for MTHFR gene are known. Variant alleles for both of these polymorphisms have been reported to reduce the enzymatic activity of the MTHFR protein. The 222Valine allele has been shown to be associated with reduced risk of a number of cancers including UBNs, while some other studies have shown an increased risk with this variant [80, 97, 115-119]. There are two possible hypotheses that describe the role of 222Valine allele in carcinogenesis. Increased cancer risks with this allele may be associated with insufficient methylation of DNA due low activity of MTHFR. While, lowered risks may be caused by the increased fidelity of DNA synthesis afforded by the greater availability of MTHFR substrate 5,10-methylenetetrahydrofolate [120]. It is also postulated that the protective or adverse effect of 222Valine allele depends on the level of dietary folate. Functional significance for the E429A (A>C) polymorphism in the MTHFR gene is not yet established. However, a decreased risk of leukemia and colorectal cancer have been reported with the variant allele of the polymorphism [121, 122].

2.4 Polymorphisms in the DNA repair genes and the risk of cancer

2.4.1 DNA repair systems

DNA is continuously exposed to a range of damaging agents, which include reactive cellular metabolites, environmental chemicals, ionizing radiation and UV light. The consequences of DNA lesions are diverse and adverse. Acute effects of DNA damage involves obstruction of transcription and replication, causing cellular malfunctioning and cell death and in the long run contributing to aging. Eventually, inaccurate repair of DNA damage results in mutations that may induce cancer. An intertwined set of genome surveillance mechanisms collectively counteract the consequences of genomic insults, including nucleotide excision repair (NER), base excision repair (BER), homologous recombination repair (HRR), non-homologous end joining (NHEJ) and mismatch repair (MMR).

Nucleotide excision repair (NER) is capable of removing UV-induced pyrimidine dimers, photoproducts, and DNA cross-links. Other NER substrates include bulky chemical adducts, DNA intra-strand cross links, and some forms of oxidative damage. The common features of lesions repaired by the NER pathway are helical distortion of the DNA duplex and a modification of the DNA chemistry [123]. The NER process requires the action of more than 30 proteins in a stepwise manner that includes damage recognition, local opening of the DNA duplex around the lesion, dual incision of the damaged DNA strand, gap repair synthesis, and strand ligation (Fig.6) [124]. There are two distinct forms of NER: global genomic NER (GG-NER), which corrects damage in transcriptionally silent areas of the genome, and transcription coupled NER (TC-NER), which repairs lesions on the actively transcribed strand of the DNA. These two sub-pathways are fundamentally identical except in their mechanism of damage recognition (Fig. 6). In GG-NER, the XPC/HR23B protein complex is responsible for the initial detection of damaged DNA (Fig. 6A). Conversely, damage recognition during TC-NER does not require XPC, but rather is thought to occur when the transcription machinery is stalled at the site of injury. The stalled transcription machinery is displaced by the action of the CSA and CSB proteins, as well as other TC-NER-specific factors (Fig. 6B). The subsequent steps of GG- and TC-NER proceed in an essentially identical manner.

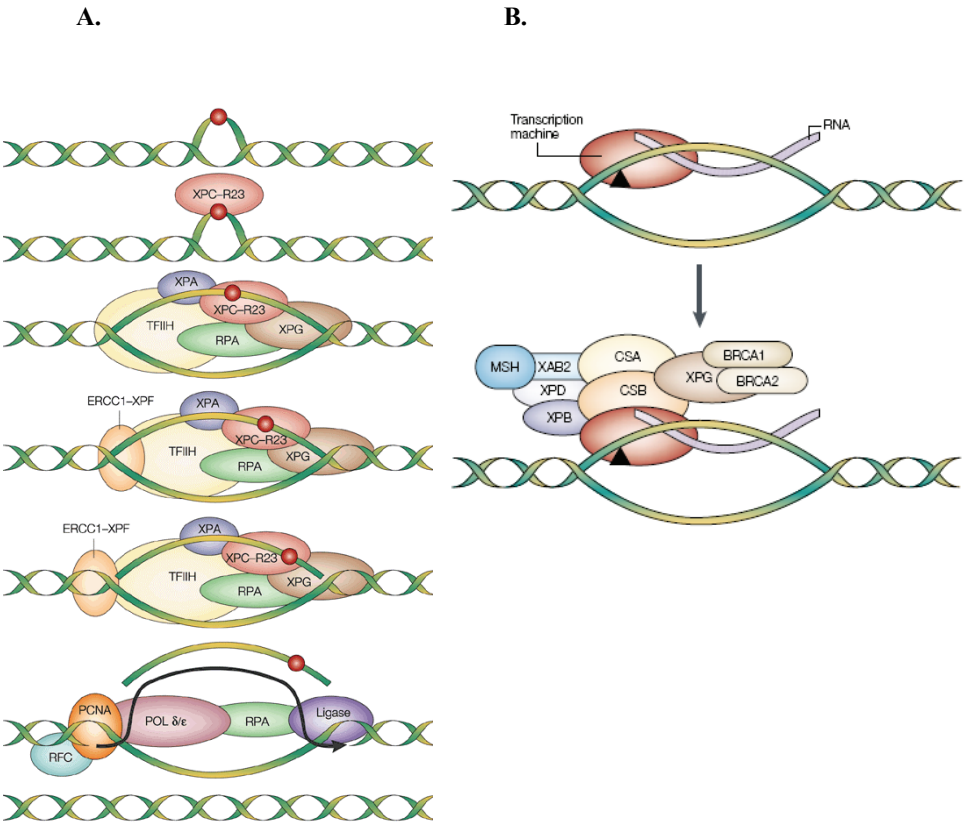

Figure 6. *Model of **Nucleotide Excision Repair**. (A) Global genomic-NER; the helical distortion is recognized by the XPC-HR23B complex. (B) Damage recognition in Transcription coupled-NER; the stalled RNA polymerase II acts as a marker for lesion recognition by CSA/CSB complex. The subsequent steps of these processes are shared and include, local unwinding of the DNA by TFIIH, a nine subunit complex of, XPB, XPD and CCNH. The un-wound DNA is then stabilized by RPA while XPA assemble other DNA repair factors. The damaged DNA is subsequently removed by a dual incision with XPG (at 3′) and ERCC1-XPF (at 5′). Finally the gap is filled by DNA polymerase δ/ε followed by a sealing with DNA ligase [Modified and reprinted by permission from Nature Reviews Cancer [125] copyright (2001) Nature Publishing Group]*.

Base excision repair (**BER)** is a multi-step process that repairs non-bulky damage to DNA bases resulting from oxidation, methylation, deamination, or spontaneous loss of the DNA base [126]. BER has two sub-pathways; “short patch” and “long patch” repair. Short patch repair replaces a single nucleotide, while long patch repair replaces approximately 2 to 10 nucleotides from the lesion. Both pathways are initiated with the modification of damaged base by DNA glycosylase and AP

Endonuclease 1 (APE1) (Fig 7). In “short patch” repair the DNA polymerase ß (Pol ß) removes this modified base by its AP lyase activity and adds one nucleotide to the 3' end of the nick (Fig. 7A) [127]. The "long-patch" repair, is employed when a modified base shows resistance to the AP lyase activity of DNA Pol ß [128]. Unlike short-patch , long-patch repair is a PCNA-dependent pathway, where the DNA polymerase (δ, or ε) adds several nucleotides to the gap (Fig. 7B) [129]. Sealing of strand nick complete the BER process (Fig 7).

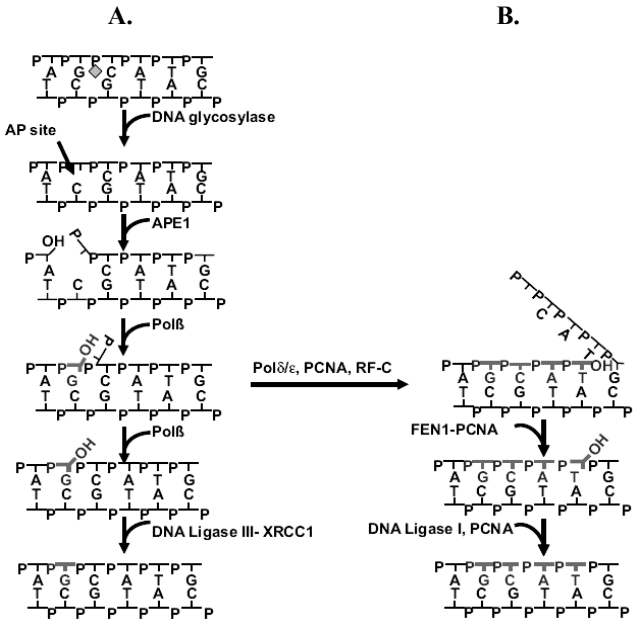

Figure 7. *Model of **Base Excision Repair**. (A) Short patch and (B) Long patch BER pathways. DNA glycosylase initiates both of these pathways by generating an apurinic/apyrimidinic (AP) site. The AP site is further processed by the APE1/APEX1 endonucleases which cleave the sugar-phosphate chain on the 5' side of the AP site and produce a deoxyribose-phosphate (dRP) residue. Following polymerase ß (Polß) adds one nucleotide to the 3' end of the nick and removes the dRP moiety via its associated AP lyase activity. The strand nick is finally sealed by a DNA ligase III/XRCC1 complex in "short-patch" repair. The long-patch repair is a PCNA-dependent pathway, where the DNA polymerase (δ or ε) adds several nucleotides to the repair gap thus displacing the dRP as part of a "flap" oligonucleotide. The resulting oligonucleotide overhang is excised by the FEN-1 prior to sealing of the nick by a DNA ligaseI [Reprinted by permission from American Journal of Epidemiology [130] copyright (2005) Oxford University Press]*

Double-strand breaks (DSBs) affect both strands of the DNA duplex and therefore prevent use of the complementary strand as a template for repair [131]. DSBs in DNA are caused by a variety of sources including ionizing radiation, certain

genotoxic chemicals, endogenously generated reactive oxygen species, replication of single-stranded DNA breaks, and mechanical stress on the chromosomes. To counteract the detrimental effects of these potent lesions, cells have evolved two distinct pathways of DSB repair, **homologous recombination** (Fig. 8A) and **non-homologous end joining** (Fig. 8B) [132]. **Homologous recombination** -directed repair corrects DSB in an error-free manner using a mechanism that retrieves genetic information from a homologous, undamaged DNA molecule. The RAD52 group of proteins, including RAD50, RAD51, RAD52, RAD54, and MRE11 mediate this process [133]. In contrast to homologous recombination, **non-homologous end joining** does not require a homologous template for DSB repair and usually results in the correction of the break in an error-prone manner. Essential to the non-homologous end joining pathway is the activity of the Ku70/Ku80 hetero-dimeric protein [134].

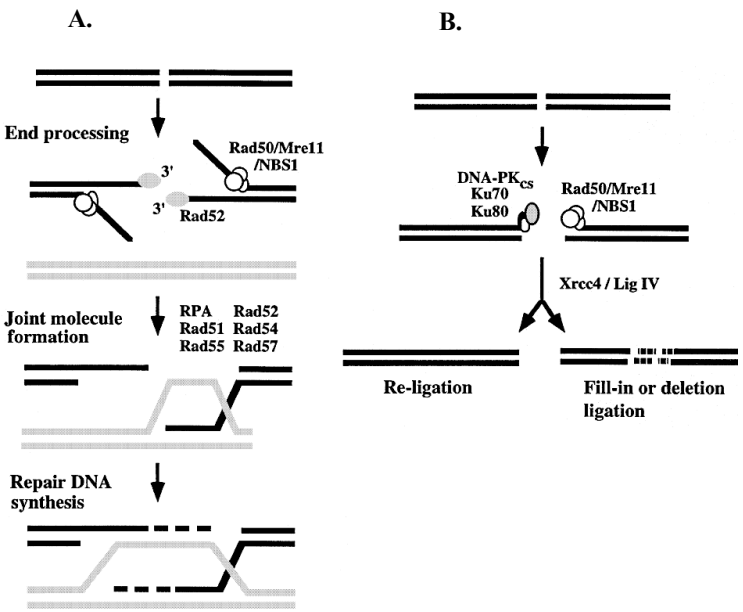

Figure 8. *Model of **Double-strand break repair**. (A) Homologous recombinational repair; initially processed by the Mre11/Rad50/NBS1 nuclease complex. Rad52 protects the end of DNA strand. Rad51 and its associated proteins are involved in the heteroduplex formation. Intact DNA from the homologous chromosome (shown in grey lines) is used as a template to replace the genetic information lost during the initial nucleolytic process. Following the nucleolytic process, Holliday junction, branch migration, nuclease resolution of the junction and ligation of the DNA complete the recombinational repair (B) Non-homologous end-joining pathway; the Ku heterodimer binds to DNA ends and recruits DNA-PKcs. The XRCC4/ligase IV complex is required for the joining of DNA ends in this repair process. [Reprinted by permission from Oncogene [135]. copyright (1999) Nature Publishing Group*

The **mismatch repair (MMR)** pathway plays an essential role in the correction of replication errors such as base-base mismatches and insertion/deletion loops that result from misincorporation of nucleotides and template slippage, respectively. The MMR pathway is initiated with the recognition of mismatch or insertion/deletion by a heterodimer of the MSH2-MSH6 or MSH2-MSH3 proteins (Fig.9) [136-138]. Following lesion identification higher order protein complexes are formed, including those containing the heterodimeric proteins of MLH1-PMS2, MLH1-MLH3, and replication factors. Excision and re-synthesis of the nascent strand is performed by a number of factors including PCNA, RPA, RFC, exonuclease I, DNA polymerases δ and ε, endonuclease FEN1, and additional factors [139].

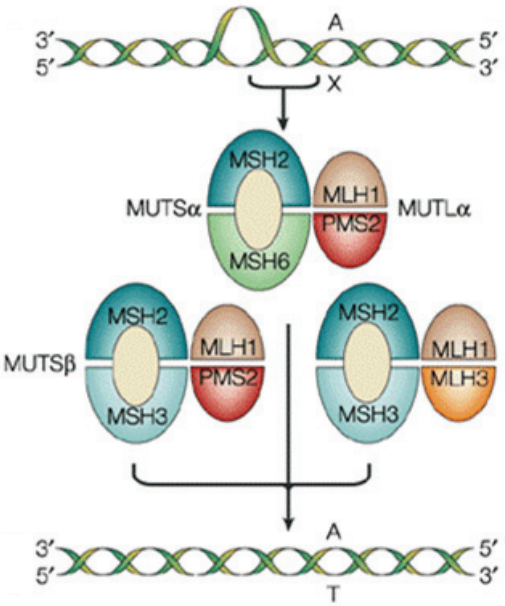

Figure 9. *Model of **Mismatch Repair.** The recognition of small loops generated by insertion or deletion of nucleotides, as well as single base mismatches (A:X) is primarily accomplished by a complex called MUTSα, a heterodimer of MSH2 and MSH6. Another heterodimer, MUTSβ, comprising MSH2 and MSH3, can also operate in the recognition of small loops during MMR. The precise biochemical events subsequent to mismatch recognition in mammalian cells are not well understood, but are believed to involve other heterodimeric complexes, comprising proteins called MLH1, PMS2 and MLH3. [Modified and reprinted by permission from Nature Reviews Cancer [125]. copyright (2001) Nature Publishing Group]*

2.4.2 Risk of cancer with different DNA repair gene polymorphisms

Polymorphisms in several DNA repair genes including XPC, XPD, XPG, XRCC1, OGG1, XRCC3, NBS1, MSH3, MSH6 are reported to be associated with the

risk of several cancers [3]. Risk of UBNs with the polymorphisms in the above mentioned DNA repair genes are summarized in table 2.

The **xeroderma pigmentosum group C (XPC)** gene spans 33 kb on chromosome 3 and contains 16 exons, encoding a 940 amino acid protein [140]. The XPC protein binds to HR23B and plays a key role in global genomic NER by damage recognition (Fig 6A) [141]. Interestingly XPC also plays an unexpected and multifaceted role in BER and protect cells from oxidative DNA damage [142]. Germ line mutations in the XPC gene result in defective NER, and are associated with the *Xeroderma pigmentosum* syndrome. Beside mutations, several polymorphisms in this gene have been reported [140, 143, 144]. A common poly (AT) insertion/deletion polymorphism in the intron 9 of XPC gene, is in linkage disequilibrium with another K939Q (A>C) polymorphism of this gene. These two polymorphisms were shown to alter the repair capacity and risk of different types of cancers including, lung cancer, squamous cell carcinoma of head and neck and cutaneous melanoma [145-147]. Another SNP at codon 499 of XPC protein which replaces an alanine for a valine is also reported to modulate the risk of lung, advanced colorectal, endometrial and urinary baldder cancer [148-151]. Haplotypes of A499V(C>T) and K939Q (A>C) polymorphisms in XPC gene have been shown to reduced the risk of UBNs [152].

The 54.3-kb **xeroderma pigmentosum group D (XPD)** gene has 23 exons and codes for an evolutionarily conserved helicase, a subunit of TFIIH that is essential for both transcription and NER (Fig 6) [153]. Mutations in the XPD gene result in a defective NER and are associated with the *Xeroderma pigmentosum* syndrome [154, 155]. Several polymorphisms in the coding region of this gene with relatively high allele frequencies are identified. The two most common SNPs in the XPD gene, which have been the subject of various case–control and functional studies, are D312N (G>A) and K751Q (A>C). The D312N (G>A) polymorphism is situated in a highly conserved region of the XPD protein and has shown to alter the UV-induced apoptosis and the risk of cancers [156-160]. The K751Q (A>C) polymorphism of XPD gene has also been found to affect the repair process and the risk of cancers including UBNs [3, 157, 161-164].

The **xeroderma pigmentosum group G (XPG)** gene codes for a protein with a predicted molecular mass of 133 kDa and is a member of the RAD2/XPG family [165]. During NER the XPG protein makes a 3′ incision at the border of the open DNA intermediate (Fig. 6) [165, 166]. It also helps to assemble a number of DNA-protein complexes in the NER process [167]. Besides, the XPG protein also couples various

repair processes to transcription [168, 169]. Among the several SNPs the D1104H (G>C) polymorphism of the XPG gene has been investigated for its association with the risk of cancer [170]. The variant allele homozygotes for this XPG polymorphism have been found to be associated with decreased risk of lung, urinary bladder and breast cancer [95, 171, 172].

Table 2. Overviews of the effect of studied DNA repair gene polymorphisms on the function of DNA repair proteins and risk of UBNs.

Genes (repair pathway)	**Common variant / Rare variant (position in codon)**	**Frequency of rare variant in Caucasian [ref]**	**Putative functional association with the rare variant**	**Ref. with respect to altered risk of UBNs**
XPC (NER)	A/V (499)	0.35	Decreased NERC [173]	Increased risk [151]
	K/Q (939)	0.34	Altered expression of XPC [174] Decreased SSB repair [162]	Increased risk [7, 95]
	PAT-/PAT+	0.44 [143]	Decreased NERC* [175]	
XPD (NER)	K/Q (751)	0.27	Decreased NERC [176] Increased CA repair [162]	Decreased risk [177] Increased risk in women [159] Improved survival after chemo-radiotherapy[12]
XPG (NER)	D/H (1104)	0.15	Decreased repair rate of TT=T dimer [172]	Increased risk [95, 178]
XRCC1 (BER)	R/Q (399)	0.47	Decreased BER capacity [179]	Decreased risk [180-182] Improved survival after radiotherapy [183]
OGG1 (BER)	S/C (326)	0.22	Decreased repair of 8-oxoguanine [184]	-
XRCC3 (HRR)	T/M (241)	0.45	Reduced repair of bulky DNA adducts and CA [179, 185]	Increased risk [186] Decreased risk [180]
NBS1 (NHEJ)	E/Q (185)	0.31	Unknown	Marginally increased risk [95]
MSH3 (MMR)	A/T (1036)	0.35	Unknown	Increased risk of high stage disease [187]
MSH6 (MMR)	G/E (39)	0.21	Unknown	Increased risk of high stage/grade disease [188]

The **x-ray repair cross-complementing group 1 (XRCC1)** gene is located on chromosome 19q13.2 and encodes a protein of 633 amino acids. The XRCC1 protein has no known enzymatic activity, and is thought to act as a scaffold protein in both single-strand break repair and base excision repair (Fig 7A) [189]. XRCC1 has been shown to physically interact with DNA polymerase ß, PARP1 and 2, APE1/APEX1, OGG1, and proliferating cell nuclear antigen. Its absence leads to a substantial reduction in the levels of its partner ligase III [190, 191]. Among the different non-synonymous SNPs identified in the XRCC1 gene the R399Q (G>A) polymorphism is located in the region of PARP1 and BRCA1 interacting domain of XRCC1 protein and been extensively investigated both for its function and association with cancer risk [130, 192, 193].

The **8-Oxoguanine DNA glycosylase (OGG1)** gene is located on chromosome 3p26.2, a region that frequently shows loss of heterozygosity in several human cancers [184, 194]. The gene contains seven exons and encodes a bi-functional glycosylase involved in base excision repair (Fig 7). OGG1 repairs 8-oxoguanine, which is able to base-pair with adenine and cause a G:C>T:A transversion in repair-deficient bacteria and yeast [194]. At least 20 validated SNPs of this gene are identified among which, the S326C (C>G) polymorphism has been studied most frequently. Several in vivo or in vitro studies have examined the association between OGG1 genotypes and enzyme activity, though the results are contradictory, as reviewed by Weiss et al. [195]. A recent meta analysis with the S326C (C>G) polymorphism of OGG1 gene showed increased risk of lung cancer in the variant allele homozygotes of this polymorphism [130].

The **x-ray repair cross-complementing group 3 (XRCC3)** gene is located on chromosome 14q32.3 and encodes a protein of 346 amino acids. The XRCC3 protein participates in the homologous recombination repair pathway and is a member of the family of Rad-51-related proteins [196-198]. During homologous recombination repair, XRCC3 is required for accumulation of RAD51 protein at the site of DNA damage and is also involved in the resolution of holiday junction at the later stages of homologous recombination repair [199, 200]. To date four non-synonymous SNPs of XRCC3 gene has been identified. The variant allele for T241M (C>T) polymorphism in the XRCC3 gene has been found to be associated with increased rate of chromosomal deletions in x-ray-challenged blood lymphocytes [201]. Moreover, the association of variant allele of this XRCC3 polymorphism with the risk of basal cell carcinoma of skin, melanoma,

head and neck, breast and bladder cancer further indicates its possible effect on the functionality of the XRCC3 protein [186, 202-205].

The **Nijmegen breakage syndrome 1 (NBS1)** gene contains 16 exons and is mapped on chromosome 8q21. The NBS1 protein (Nibrin) is a component of the MRE11/RAD50/NBS1 complex (MRN) which is thought to participate in both non-homologous end joining and homologous recombination repair during the initial step of double strand break recognition (Fig 8) [206, 207]. NBS1 also interacts with γ–H2ax to activate the ATM kinase which in turn activates the cell-cycle checkpoint response [208]. Mutations in NBS1 cause a chromosomal instability syndrome called Nijmegen breakage syndrome [209]. The E185Q (G>C) polymorphism of the NBS1 gene is located in the breast cancer C terminal (BRCT) domain which facilitates the interaction between NBS1 and BRCA1 proteins [210]. The association of this polymorphism with the risk of breast and lung cancer have been reported [211, 212]. The variant allele of this polymorphism has also been found to be associated with a higher frequency of G:C>T:A transversion mutation of the p53 gene [213].

The **mutS homolog 3 (MSH3)** gene is located on chromosome 5q11 and encodes a protein of 1137 amino acids. The MSH3 protein binds with the MSH2 protein to form a heterodimeric complex (MutSβ) which recognizes 1-4 bp insertion/deletion loops during mismatch repair [136]. Unlike the other mismatch repair genes, mutations of MSH3 are rare in hereditary non-polyposis colorectal cancer (HNPCC) [214]. In different cancer genetic alterations reported in this gene include LOH and frame shift mutation. Besides a 9 base pair repeat polymorphism, the MSH3 gene also contains various polymorphisms [215-217]. A recent report by Kawakami et al. suggested inactivation of MSH3 gene in UBNs. They have also reported an increased risk of high stage bladder tumor with the variant allele of A1036T (A>G) polymorphism in MSH3 gene [187].

The **mutS homolog 6 (MSH6)** gene is mapped on chromosome 2p21.6 and encodes a protein of 1360 amino acids. The MSH6 protein forms a heterodimeric complex (MutSα) with the MSH2 protein which plays a key role in mismatch repair by recognizing the base–base mismatches and small insertion–deletion loops [166, 218, 219]. Germline mutations in MSH6 are responsible for 10 percent cases of HNPCC, which is associated with a tumor phenotype featuring micro satellite instability [220]. Beside mutations, several SNPs are also identified in the MSH6 gene. However, the association of MSH6 gene polymorphisms with the risk of cancer is less well studied and so is its impact on the functionality of the MSH6 protein [221].

2.5 Polymorphisms in the growth signaling and cell-cycle regulatory genes and the risk of cancer

Mammalian cells usually remain in a quiescent (G0) state and proliferate only in response to extra cellular growth signals, such as growth factors and hormones. Diverse membrane bound and cytoplasmic molecules transduce these growth signals from the cell surface to the nucleus. Growth signals promote cell division via a tightly regulated and timely coordinated process, generally referred to as cell-cycle. The eukaryotic cell-cycle is divided into four phases, the synthesis (S) phase where chromosomes are replicated, the mitotic (M) phase where the cellular components are segregated into two daughter cells and the two gap (G) phases that precede the S-phase (G1) and the M-phase (G2) [222]. Cell-cycle progression stops at two checkpoints, G1/S and G2/M, unless certain criteria are met [223]. Progression of cell-cycle beyond a check point is tightly regulated by a number of regulatory proteins. Unlike normal cells, a cancer cell proliferates in the absence of growth signals and often lose the two cell-cycle check points [1]. A lose in cell-cycle check point promotes carcinogenesis by clonal expansion of cells carrying mutation in critical genes. Independence to growth signal in cancer cells results from alterations in signal transducer protein molecules. Similarly, alterations in the proteins that regulate the cell cycle check points allow uncontrolled replication. Polymorphisms that alter the function of signal transducer or cell-cycle regulatory proteins can modulate cellular proliferation and thus alter the risk of cancer.

2.5.1 H-ras

The Harvey rat sarcoma viral oncogene homolog (H-ras) is one of the human *ras* proto-oncogenes, which encode small guanine binding proteins (p21ras) with intrinsic GTPase activity [224, 225]. These membrane bound Ras proteins transduce intracellular signals implicated in diverse biological processes including cellular proliferation and transformation, via association with multiple effecter proteins [226]. The major pathway through which ras mediates its signal is the Ras-Raf-Map-Kinase pathway (Fig.10). The H-ras gene is located on chromosome 11 and alternatively spliced to produce two alternative transcripts [227-229]. Mutations in H-*ras* gene that constitutively activate the H-ras proteins occur in nearly 30 percent of all human tumors including tumors of bladder [230, 231]. In addition to mutations,

inherited polymorphisms in the H-ras gene were described [232, 233]. The silent H27H (T>C) polymorphism of the H-ras gene were shown to be associated with the risk of UBNs (Table 3) however, the mechanisms by which this silent H-ras polymorphism modifies the risk of UBNs is unknown [9, 95, 188]. But it is conceivable that this SNP is linked to other functional polymorphic sites in non-coding regions of H-ras.

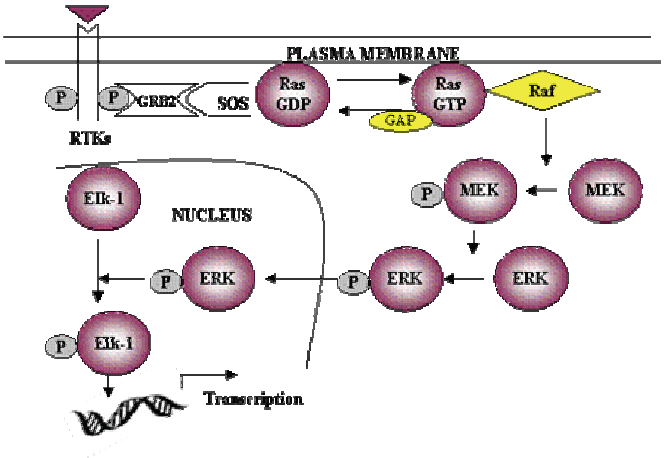

Figure 10. *Summary of signaling through Ras-Raf-MAPK pathway. Receptor tyrosine kinase become phosphorylated upon binding to a ligand. This results in the activation of Ras and subsequently the phosphorylation of ERK. The phosphorylated ERK translocates to the nucleus where it initiates transcription via activation of transcription factors such as Elk-1.*

2.5.2 Cyclin D1

Since enhanced cell proliferation is a property of human cancer by definition, altered cell cycle regulation seems a prerequisite for cancer development. The mitotic cell-cycle is powerfully controlled by the cyclin D1 protein (also called PRAD1 or CCND1), which plays an important role in the transition from the G1 phase to the S phase of the cell-cycle [11]. The predominant action of cyclin D1 in cell cycle control is believed to be manifested via its interactions with cyclin dependent kinase 4/6 (CDK4/CDK6) protein [234]. Upon activation the CDK4/CDK6 protein initiates phosphorylation of the retinoblastoma (RB) protein. In the hypo-phosphorylated state, RB inhibits cell cycle progression through its ability to repress transactivation of genes required for DNA replication and G2/M progression. However phospholrylation of RB by cyclin D1-CDK4/CDK6 complex disrupts its ability of transcriptional repression and therefore nullifies its anti-proliferative function [235, 236]. The cyclin D1-CDK4/CDK6 complexes are tightly regulated by the $p16^{INK4a}$ which binds to the CDK-moiety and disrupts its association with cyclin D1 [237] (Fig 11).

Eexcessive cyclin D1 expression and/or activity is common in human cancers [238-241]. Although somatic mutations of the cyclin D1 locus are rarely observed, mounting evidence demonstrates that the P241P (G>A) polymorphism of cyclin D1 gene is related to its alternate splicing and the risk of cancer [242]. The variant nucleotide interferes with splicing because of its unique localization within a conserved splice donor region. The G allele splices the transcript 'a' while the A allele splices transcript'b'. The protein encoded by the 'b' transcript has a longer half-life and has been shown to accelerate the S phase entry. It has therefore been suggested that DNA damage in cells with the A allele may bypass the G1/S checkpoint more easily than the cells with G allele [243]. The A allele has also showed association with the risk of UBNs as shown in table 3.

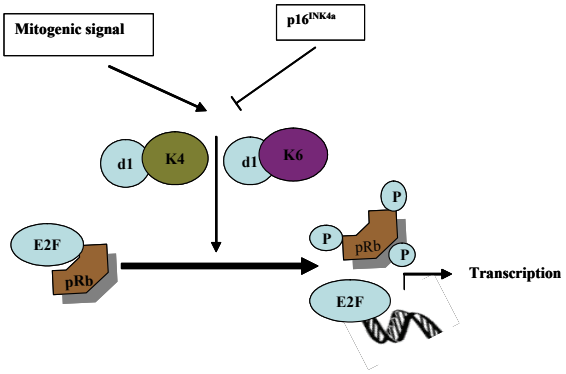

Figure 11. *Regulation of G1/S transition. Mitogens induce assembly of cyclin D1-CDK4/6 complexes which in turn phosphorylate RB. Phosphorylated RB release E2F to start transcription and the cell moves from G1 to S phase. The action of cyclin D1 is antagonized by p16^{INK4a} which binds to the CDK-moiety.*

Table 3. Overviews of the effect of studied H-ras and cyclin D1 gene polymorphisms on the function of their proteins and risk of UBNs.

Genes	**Common variant / Rare variant (position in codon)**	**Frequency of rare variant in Caucasian**	**Putative functional association with the rare variant**	**Ref. with respect to altered risk of UBNs**
H-ras	H/H (27)	0.38	Unknown	Increased risk [9] Decreased risk [95]
Cyclin D1	P/P (241)	0.50	Increased half life of the protein Increased S-phase entry	Increased risk [244, 245]

3. THE PRESENT STUDY

3.1 Aim

The general aim of this thesis was to investigate the role of polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes in the susceptibility, pathogenesis and clinical behavior of urinary bladder neoplasms. The specific objectives of the study were:

1. To analyze the effect of genotypes, haplotypes and combined genotypes of DNA repair, metabolic and cell-cycle regulatory gene polymorphisms on the susceptibility to urinary bladder neoplasms. (Study I and II)

2. To investigate the influence of polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes on disease course and clinical outcome in patients with urinary bladder neoplasms. (Study III and IV)

3.2 Design

In the present thesis "**association studies"** were performed to investigate the relation between genetic polymorphisms and urinary bladder neoplasms. Association studies are potential approaches for the detection of susceptibility alleles [246]. Two kinds of association studies namely, case-control and cohort studies have been used for the present investigation.

3.2.1 Case-control study

This is an epidemiological study in which subjects are selected according to their disease status (e.g. with and without disease) and further classified according to their exposure status to calculate the risk of disease with the exposure. For the present thesis, case-control studies were performed to analyze the association of genotypes, haplotypes and genotype combinations of studied polymorphisms with the susceptibility to urinary bladder neoplasms (Fig. 12A).

3.2.2 Cohort study

By definition cohort study is an epidemiological study in which a particular outcome is compared in groups of people who are similar in most ways but differ by a certain characteristic (exposure). For this thesis, cohort studies were performed to investigate the influence of studied polymorphisms on disease course and clinical outcome in patients with urinary bladder neoplasms (Fig. 12B).

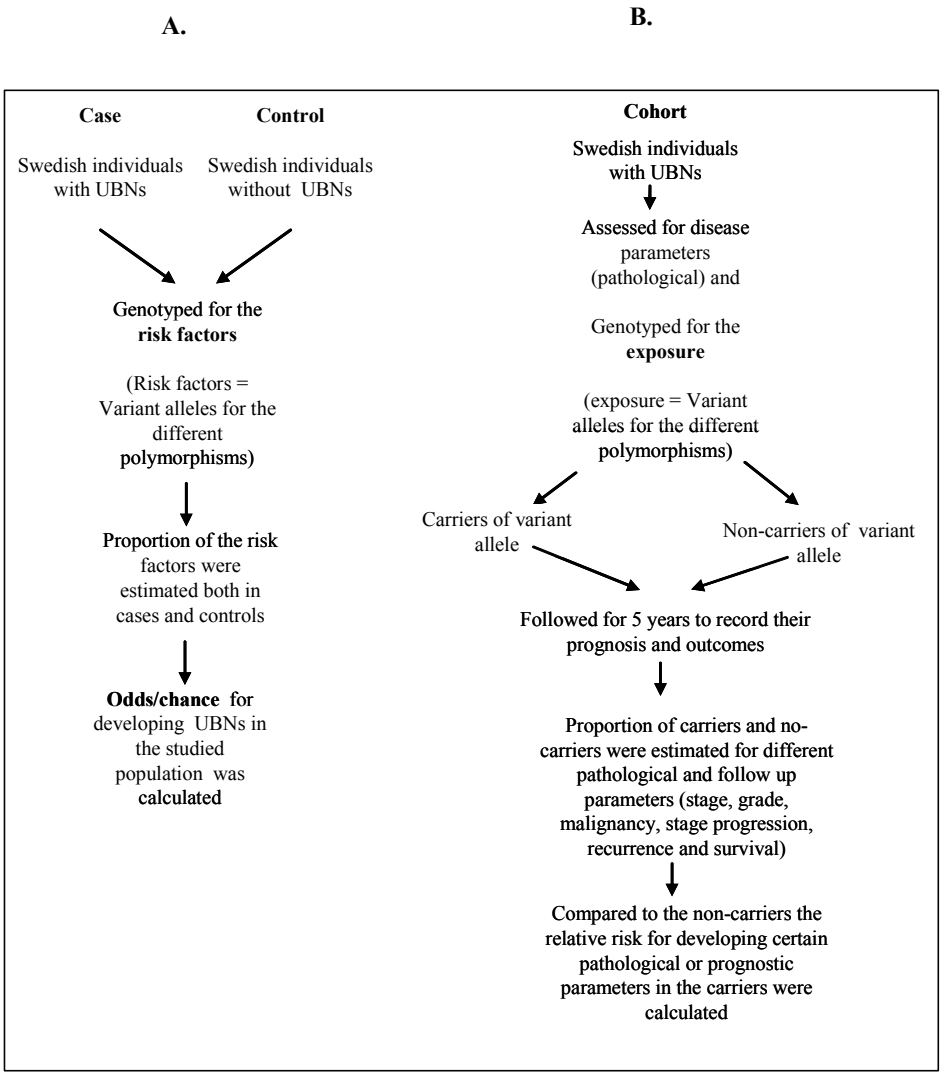

Figure 12. *Study design. A. Case-control study (paper I and II) and B. Cohort study (paper III and IV).*

3.3 Materials - The study population

Study population used for the present thesis comprised of cases with urinary bladder neoplasms and healthy controls from Sweden. **The cases** were taken from a population based material of 546 patients with newly diagnosed **urinary bladder neoplasms.** Patients were recruited in the urology clinic of seven different hospitals in the Stockholm county area during the years 1995 and 1996 and represented almost all new cases of UBNs within the area during that period. The demographic and exposure data about the patients were obtained from a structured questionnaire which was answered by about 50 percent of the participants. The mean age of the patients at diagnosis was 69.6 years (median 71; range 34-96). Follow-up information on stage progression, recurrence, and survival of studied patient population were recorded for 5 years with the mean follow-up (n=295) of 46.3 months (range 1-75 months). Patients were subdivided into five different disease categories (TIS, TaG1, TaG2, TaG3+T1 and T2+) depending on the clinical outcomes and micro-pathological characteristics (data unpublished) of the tumors [20]. Out of 546 patients, blood samples were collected from 354 before commencement of any treatment, and used for the studies included in this thesis. **The controls** (n=324) were chosen from the same geographical area, ethnic background and broadly from the similar age groups as the cases. The mean age of the controls was 52.4 years (median 51; range 22-89). Genomic DNA was extracted from the blood samples of case and control subjects.

3.4 Methods

3.4.1Genotyping

Genomic DNA extracted from the blood samples of cases and controls were genotyped for different polymorphisms in the metabolic, DNA repair and cell-cycle regulatory genes with the following methods:

3.4.1. a. PCR-RFLP

This method of genotyping was employed when a SNP either abolished or created restriction site/s for a particular restriction enzyme and therefore changed the length of a restriction fragment. The DNA fragment of interest with the SNP was amplified with PCR technique. This was followed by a restriction digestion of the amplified fragments with appropriate restriction endonucleases which specifically recognized and cut either the variant or the common sequences. Finally, the digested

products were resolved by polyacrylamide gel electrophoresis and stained with ethidium bromide for visualization under UV light (Fig 13). Absence of contaminations was checked by including a negative control. The cutting efficiency of the enzymes was also standardized to avoid misinterpretation of genotype due to partial digestion.

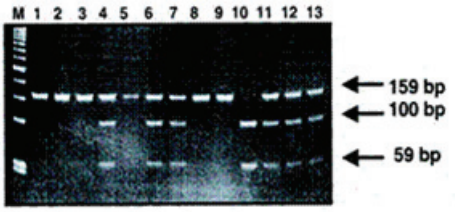

Figure 13. *Migration of fragments of exon 15 of the XPG gene after restriction digestion of PCR amplified products with Hsp92II, on a polyacrylamide gel. Lanes 1, 2, 3, 5, 8 and 9 contain only uncut wild-type 159 bp fragment. Lanes 4, 6, 7, 11, 12 and 13 contains both uncut wild-type and smaller 100 and 59 bp fragments from wild-type and polymorphic alleles (heterozygotes), respectively. Lane 10 contains only smaller fragments due to polymorphic base change in both alleles (homozygote).*

3.4.1. b. PCR and duplex PCR

PCR and duplex PCR followed by a direct gel electrophoresis of the PCR products were employed to determine the genotypes for insertion/deletion and homozygous deletion polymorphisms, respectively. To genotype insertion/deletion polymorphisms, amplified PCR products were directly resolved on a polyacrylamide gel and differentiated according to their size. To genotype homozygous deletion polymorphisms the genes under investigation were co-amplified with a fragment of another gene which served as an internal control. The amplified PCR products were further analyzed by gel electrophoresis

3.4.1. c. Taq-Man allelic discrimination method

In this method, fluorogenic allele-specific probes, labeled with both fluorescent reporter (VIC or FAM) and quencher dyes were used in the PCR assay. Each probe annealed specifically to their complementary sequences between the forward and reverse primer recognition sites. During the PCR extension reaction the 5′ exonuclease activity of the TaqDNA polymerase excised the quencher dye from the hybridized probe which allowed the reporter dye to emit its fluorescence. Following, excision the probe was removed from the DNA template, whereby polymerization continued. The fluorescence signal(s) generated by PCR amplification indicated the nature of the alleles that were present in the sample (Fig 14). The assay was performed under competitive conditions with both probes present in the same reaction.

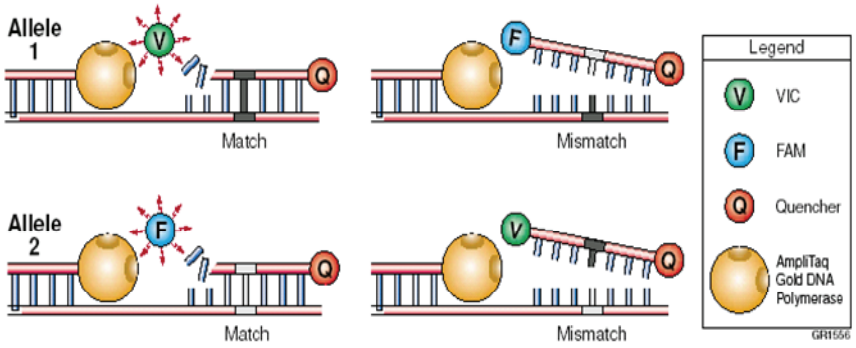

Figure 14. *The probe for allele 1 and allele 2 are respectively labeled with V and F dye. The fluorescence of the V or F initially remains silenced by the quencher Q. During the extension phase of the PCR cycle the AmpliTaq Gold DNA polymerase cleaves the reporter dye from the probe with its 5′>3′ exonuclease activity. After being separated from the quencher, the reporter dye emits its characteristic fluorescence. Therefore, a substantial increase only in V dye fluorescence indicates homozygosity for allele 1. Similarly, a substantial increase only in F dye fluorescence indicates homozygosity for allele 2. Increase in both signals indicates heterzygosity for allele 1 and allele 2.*

3.4.2 Sequencing

The genotype results were regularly checked and confirmed by automated sequencing of amplified fragments. Sequencing was carried out using a dye terminator kit (Big Dye, Applied Biosystems) and an automated sequencer (ABI 377, Applied Biosystems). The sequences obtained from sequencing reactions were aligned with the Gene-bank sequences using the Align software in the DNAStar package (DNAStar Inc., Madison WI).

3.4.3 Statistical analyses

Chi-square test, Fisher's exact test, Cox proportional hazards model and Kaplan-Meier method were used to study the association of bladder neoplasms with the genotypes from the different polymorphisms studied. The haplotypes and linkage disequilibrium were determined using a Haploview program (version2.5, http://www.broad.mit.edu/personal/jcbarret /haploview/).

3.5 Summary of the results

3.5.1 Paper I- Polymorphisms in DNA repair and metabolic genes in urinary bladder neoplasms

A number of previous reports tempted us to hypothesize that genetic polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes may influence the risk of UBNs in a given population [177, 181, 186, 244, 247]. The present study was therefore set to investigate the relation between genetic polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes and the risk of UBNs in patients recruited in hospitals from the Stockholm county area during 1995-96. We have investigated 12 polymorphisms in 11 different genes (XPC, XPD, XPG, XRCC1, XRCC3, NBS1, NQO1, MTHFR, GSTT1, H-ras, and cyclin D1) in the cases of UBNs and matching controls in order to evaluate their effect on susceptibility to urinary bladder neoplasms.

Our results demonstrate that amongst the DNA repair genes, the variant allele of K939Q (A>C) polymorphism in the XPC gene was associated with an increased risk of UBNs. The frequency of variant C allele for the K939Q (A>C) polymorphism was found to be significantly higher in the cases than in control population (p=0.001) and the carriers of this allele had an increased risk for UBNs (OR 1.49, 95% CI 1.16-1.92). Moreover, individuals homozygote for the variant allele of K939Q (A>C) polymorphism exhibited almost 2 fold increased risk for this disease (Fig. 15). To our knowledge, this was the first case-control study for this exonic polymorphism in the XPC gene.

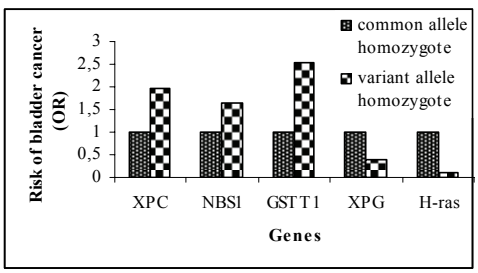

Figure 15. *Risk of urinary bladder neoplasms in the variant allele homozygotes of different DNA repair, metabolic and cell cycle regulatory gene polymorphisms.*

Individuals homozygous for the variant allele of E185Q (G>C) polymorphism in the NBS1 gene were also at increased risk for UBNs. In contrast, a decreased risk for UBNs was observed in the variant allele homzygotes of D1104 (G>C) and H27H (T>C) polymorphisms in the XPG and H-ras gene, respectively. Among the metabolic

genes, an association was found between the occurrence of urinary bladder neoplasms and the null allele homozygote of GSTT1 gene (Fig. 15). However, when Bonferroni correction for multiple comparisons was taken into consideration, only the association of UBNs with the XPC and GSTT1 gene remained significant. For other polymorphisms included in this study no significant differences for genotype distributions and allele frequencies between the cases and the controls were observed.

3.5.2 Paper II-XPC genotype/haplotype and the risk of urinary bladder neoplasms

Our previous work, that demonstrated a strong association between the K939Q (A>C) polymorphism in the XPC gene and the risk of urinary bladder neoplasms served as a motivation for the present study in which, we have investigated the association of two more, (A499V;C>T and PAT; -/+) polymorphisms in the XPC gene with the risk of UBNs in patients recruited in hospitals from the Stockholm county area during 1995-96 [95]. We determined the frequency of A499V (C>T) and PAT (-/+) polymorphisms in the cases of UBNs and matching controls in order to evaluate their effect on risk modulation. We also tested the linkage disequilibrium (LD) between the K939Q (A>C), A499V (C>T) and PAT (-/+) polymorphic loci in the XPC gene and investigated the association of their haplotypes and combined genotypes with the risk of UBNs.

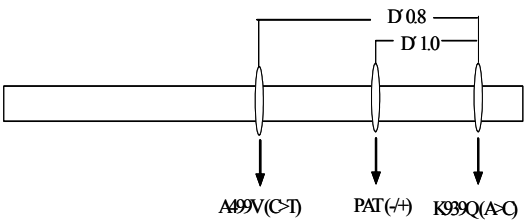

Figure 16. *Coefficient of linkage disequilibria (D') between the A499V (C>T), K939Q (A>C) and PAT (-/+) polymorphic loci in the XPC gene.*

As shown in figure 16, the K939Q (A>C) and PAT (-/+) polymorphisms were in total linkage and therefore, we chose the K939Q (A>C) as a marker for both. An increased risk of UBNs was observed in the variant allele homozygotes of A499V (C>T) polymorphism (OR 5.7, 95% CI 3.4-9.5). The variant allele homozygotes for the A499V polymorphism also showed association with earlier age of the disease onset compared to patients homozygous for the common allele (*P* 0.004). Each of the variant allele haplotypes for A499V (C>T) and K939Q (A>C) polymorphisms in the XPC gene were associated with enhanced risk of this disease (Fig. 17 A). The combined

genotype analysis showed an increased risk of the disease with the increasing number of variant alleles (Fig. 17 B). This analysis also revealed that the carriers of variant allele for the A499V (C>T) polymorphism were at 2.5-fold increased risk while the K939Q (A>C) variant allele carriers were at 1.6-fold increased risk compared to individuals homozygote for both polymorphisms.

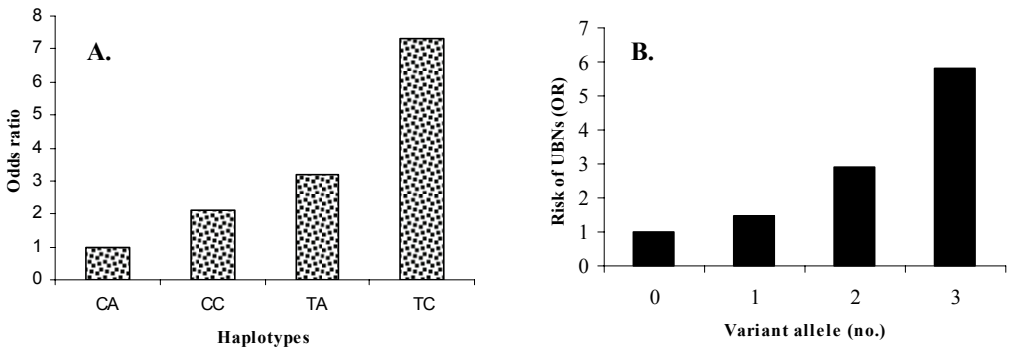

Figure 17. *Risk of UBNs with: (A). haplotypes and (B). number of variant alleles in the genotypes constructed from the A499V (C>T) and K939Q (A>C) polymorphisms in the XPC gene.*

3.5.3 Paper III-Influence of polymorphisms in DNA repair and cell-cycle regulatory genes on the outcomes of urinary bladder neoplasms

Besides being risk modulators during early stage of carcinogenesis, genetic polymorphisms can also potentially alter the clinical course and disease outcomes [58]. We therefore investigated the association of polymorphisms in DNA repair and cell-cycle regulatory genes with the clinical outcomes of UBNs. To such end, a follow-up study was conducted with the same cancer population used for the two previous studies. The follow-up parameters of each cancer patient were correlated later with their genotypes for different DNA repair and cell-cycle regulatory gene polymorphisms.

Patients carrying the variant allele of K751Q (A>C) polymorphism in the XPD gene were found to be at lower risk of death (p=0.04) than the non-carriers. Compared to the non-carriers, a marginal reduction in the risk of death due to UBNs was also observed in patients carrying the variant allele for K939Q (A>C) polymorphism in the XPC gene (p=0.09). As XPC and XPD functionally interact with each other in the nucleotide excision repair pathway, we combined these two polymorphisms to check their combined effect on patients' survival. Results showed that the patients, who were

simultaneous carriers of variant alleles for the K751Q (A>C) and K939Q (A>C) polymorphisms in the XPD and XPC genes, respectively had longer disease free survival than the rest of the patients. (p=0.001, Fig. 18).

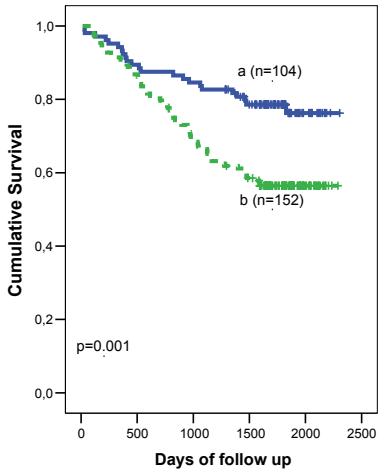

Figure 18. *Kaplan-Meier analysis for overall survival of patients with urinary bladder neoplasms; (a) simultaneous carriers of variant alleles for the K939Q (A>C) and K751Q (A>C) polymorphisms in the XPC and XPD gene, respectively; and (b) all other patients.*

The XPD polymorphism was also found to interact with the G39E (G>A) polymorphism in the MSH6 gene in modulating death of the patients. At the time of diagnosis the variant allele of G39E polymorphism in the MSH6 gene showed association with increased risk of developing highly malignant disease (TaG3+T1; p=0.03). In contrast to that, carriers of the variant allele for H27H (T>C) polymorphism in the H-ras gene were often diagnosed with low grade superficial papillary tumors (TaG1; p=0.02) and were at lower risk for stage progression (p=0.03) than non-carriers. Another intriguing finding of this study was the modulation of treatment response by the R399Q (G>A) and S326C (C>G) polymorphisms in the XRCC1 and OGG1 genes, respectively. Compared to the non-carriers, the carriers of variant allele for XRCC1 polymorphism showed lower risk for recurrence (TaG2; p=0.05) and death (T2+; p=0.03) after instillation (BCG + MMC) and radiotherapy. After radiotherapy, an inverse association of the variant allele of OGG1 polymorphism was observed with the risk of death (T2+; p=0.04). However, when Bonferroni correction for multiple comparisons was taken into consideration, only the risk of death due to combined XPC and XPD polymorphisms remained significant.

3.5.6 Paper IV- Influence of polymorphisms in metabolic genes on the outcomes of urinary bladder neoplasms

Polymorphisms in the xenobiotic metabolic genes reportedly modulate susceptibility to urinary bladder neoplasms [75, 81, 83, 86, 89]. Results of our paper I also revealed such an association [95]. Interestingly, polymorphisms in xenobiotic metabolic gene can also affect the clinical course and outcomes in the disease. The present study was therefore set to investigate the relation between genetic polymorphisms in metabolic genes and the clinical outcomes of UBNs. The study population and the study design were the same as in study III.

This study demonstrates that the NQO1 polymorphisms influenced the clinical course and outcomes of UBNs in a number of ways such as; the variant allele carriers of P187S (C>T) polymorphism in the NQO1 gene were at higher risk for high stage disease than the non-carriers at diagnosis (RR=1.4, 95%CI 1.0-1.8). Compared to the non-carriers the variant allele carriers of R139W (C>T) polymorphism in the NQO1 gene showed shorter disease-free survival in T1 disease (p=0.05). A higher risk of recurrence was also documented in the variant allele carriers than the non-carriers for this NQO1 polymorphism (p=0.03). Beside NQO1 a higher risk for highly malignant disease (T2+) was also observed in the variant allele carriers than non-carriers of I105V (A>G) polymorphism in the GSTP1 gene (RR=1.6, 95%CI 1.1-2.5). We also observed that, the carriers of variant allele for A222V (C>T) polymorphism in the MTHFR gene showed significantly longer disease-free survival than the non-carriers (p=0.02). Polymorphisms in metabolic genes are known to alter the treatment response in cancer patients, however, none of the metabolic gene polymorphisms included in this study showed any direct effect on treatment-response of UBN patients.

3.6 General discussion – The strength and limitation

The results from the present thesis are consistent with the notion that genetic polymorphisms in DNA repair, metabolic and cell cycle regulatory genes can modulate the risk for UBNs. Further, these polymorphisms can also influence the disease course and clinical outcomes in this disease. Genetic polymorphisms in these genes are considered as the main risk modulators of cancer and therefore perspective observations documented in this thesis assume importance [3, 6, 11, 58]. Further studies, including the functional consequences of the polymorphisms described in the present thesis may provide better strategies for screening and clinical care in UBNs. However, association study included here has several salient features including strengths and limitations at the same time.

In paper I and II we have conducted case-control studies to investigate the association between the genetic polymorphisms and susceptibility to UBNs. Case-control studies have been the most widely used strategy for characterizing the genetic contributions to a disease, however; a particular difficulty with this methodology is the choice of an appropriate control population. The selection of controls is crucial because any systematic allele frequency differences between cases and controls can appear as disease association, even if they only reflect the results of migratory history, gender or age differences, or other independent exposures. To minimize the exposure difference between the cases and controls the control populations in our studies were chosen from the same geographical area, ethnic background and broadly from the similar age groups as the cases. However, as we have used community based control there is a chance for recall bias in this group [248]. Using patients of other diseases as control could be a solution for that providing; the exposure/s under investigation (e.g. polymorphism/s) is not associated with the disease of the control group. To maximize similarity with disease population the control group derived from several control populations is generally recommended for ideal case-control studies [249].

In spite of the above mentioned limitations our study had a power of 90 % to detect a 2.5-fold increase of the relative risk assuming a 10 % prevalence of the rare allele in the control group. Besides, effective methods of genotyping also impart high degrees of precision to our results. Finally, polymorphisms with functional relevance in the relevant genes were included and to such end we have interpreted our observations with the possible underlying biology. The main finding of these two

studies was the association between A499V (C>T) and K939Q (A>C) polymorphisms in the XPC gene and the risk of UBNs. These XPC polymorphisms have been reported to alter the DNA damage repair capacity and are also found to be associated with the susceptibility to several cancers [7, 148, 150-152, 162, 173, 175].

Paper III and IV comprised of cohort (case only) studies and were focused to identify the clinical outcomes in the patients of UBNs in relation to the polymorphisms in DNA repair, metabolic and cell cycle regulatory genes. Cases used were clinically and pathologically well defined to stratify the risk in different disease groups [20]. However, the sample size used was small for such stratification and that calls for a cautious interpretation of our results. Moreover, due to partial availability of the exposure data, such as smoking or dietary habit of the patients we were unable to study the effect of gene-environment interaction on the development of UBNs as well as its outcomes.

The outcomes in the disease are also influenced by the treatments and the age and gender of an individual and can mask or influence the actual effect of polymorphisms on the disease [250]. Therefore, to increase the precision of our observations we have adjusted our results for such confounders. These studies demonstrated the association of polymorphisms in the XPD, XPC and NQO1 genes with the clinical course and outcomes of UBNs. The simultaneous carriers of variant alleles for both XPD and XPC polymorphisms were at significantly lower risk of death than the rest of the patient population. Polymorphisms in XPC and XPD have also been found to interact to modify risk of lung cancer [251]. It is possible that the variant alleles from the XPD and XPC polymorphisms affect a better survival in UBNs by enhanced apoptosis and growth arrest [252, 253]. Similarly, the association of NQO1 variants with high malignant disease and high risk of death and recurrence in patients with UBNs, as found in our study, is also biologically plausible. The variant allele for the NQO1 (P187S) polymorphism is associated with a reduced enzymatic activity and protein levels in bladder tumors [30]. It is likely that a reduced detoxification of carcinogens by variant NQO1 protein could result in increased DNA adduct levels in the target tissues and eventual mutations in the critical genes leading to high grade malignancy.

As multiple comparisons were carried out in study I, III and IV there were chances for making Type I error (false positive findings). To account for the use of

multiple comparisons and thus to avoid Type I error we have adjusted the P values with Bonferroni correction. However, there is an ongoing debate about the application of Bonferroni correction in biomedical research [254]. While the Bonferroni adjustment certainly decreases chances of making a Type I error, chances of making a Type II error (false negative findings) are inflated. Moreover, Bonferroni correction has limited applications in our studies as we had a pre-established hypothesis that the studied polymorphisms influence the risk as well as the clinical outcome in urinary bladder neoplasms [254]. Therefore we assumed equal importance for all of our positive findings although some of them were not significant after Bonferroni correction.

3.7 Conclusions

The main focus of this thesis was to analyze the role of polymorphisms in DNA repair, metabolic and cell-cycle regulatory genes in the susceptibility, pathogenesis and clinical behavior of urinary bladder neoplasms. Our results highlight a number of possible associations between different polymorphisms studied and the risk of UBNs. The main findings are as follows:

Risk of UBNs with the studied DNA repair gene polymorphisms

- The genotypes and haplotypes of the A499V (C>T) and K939Q (A>C) polymorphisms in the XPC gene were associated with the risk of UBNs. The risk of disease increased with the increasing number of variant alleles from these two XPC polymorphisms.
- The K751Q (A>C) polymorphism in the XPD gene either individually or in combination with K939Q (A>C) polymorphism of the XPC gene modulated the risk of death in the patients with urinary bladder neoplasms.
- The G39E (G>A) polymorphism in the MSH6 gene affected the degree of malignancy in the patients with this disease.
- The R399Q (G>A) polymorphism in the XRCC1 gene influenced the treatment response in patients with urinary bladder neoplasms.

Risk of UBNs with the studied metabolic gene polymorphisms

- The GSTT1 null allele genotype was associated with the increased risk of UBNs.
- The P187S (C>T) polymorphism in the NQO1 gene showed association with the stage and grade of UBNs at diagnosis. While, the other, R139W (C>T) polymorphism of this gene modulated the risk of death in the patients with T1 disease.
- Risk of death in patients with UBNs was also modulated by the A222V (C>T) polymorphism in the MTHFR gene.

Risk of UBNs with the studied cell-cycle regulatory gene polymorphisms

- The variant allele homozygotes for the H27H (T>C) polymorphism in the H-ras gene were at decreased risk of UBNs. Carriers of variant allele for this polymorphism also showed reduced risk for stage progression.

4. FUTURE PERSPECTIVE

Our results suggest avenues for further research in the following fields:

- The susceptibility to cancer is possibly modulated by the synergistic interaction between many polymorphisms, where each polymorphism contributes differently to the risk of disease. Therefore, to systematically examine the effect of polymorphisms on the UBNs and so as to make a genetic profile for this disease, studies on the joint impact of most relevant genetic polymorphisms can be planned.
- Polymorphisms in DNA repair and metabolic genes possibly influence the frequency as well as the type of mutations in several disease genes. We have already investigated the association of DNA repair and metabolic gene polymorphisms with the mutation spectra of p53 gene in bladder cancer patients [45, 46]. Further studies can be planned to investigate the associations of the studied polymorphisms with the mutation spectra of FGFR3 gene as well as with the other genetic alterations in the bladder tumor tissues. Such investigations will help to identify the susceptibility markers for aggression or non-aggression in bladder neoplasms.
- However, before using a polymorphic marker in the clinical set-up it is important to study the impact of that polymorphism on the function of its protein. Studies can be planned to investigate the effect of a relevant polymorphism on the expression of the respective gene both at the level of its RNA and protein.
- Polymorphisms in DNA repair and metabolic genes interact with treatments such as chemo and radiotherapy and modulate the treatment response by altering cell survival and apoptosis. Investigation about the effect of these polymorphisms on apoptosis and survival of cancer cells will help to distinguish the treatment responders and the non-responders.
- The effect of associated polymorphisms on the global gene expression can also be performed to identify the molecular signature for occurrence and progression in urinary bladder neoplasms.

5. IMPLEMENTATION

Since routine genotyping of all persons is now feasible, genetic profiling may soon become reality, and molecular and clinical epidemiological studies will have to provide the basis for understanding how to use such genetic profile for the benefit of the population. The findings of the present study constitute only a preliminary step in that direction and are long way off from any clinical application. However, with the help of further future investigations our results could be implemented into the following applications:

- Screening of individuals who are at higher risk of UBNs.
- Construction of genetic profile for the risk of UBNs.
- Differentiation of patients with aggressive and non-aggressive UBNs.
- Prediction of outcome in patients with UBNs.
- Prediction of responders and non-responders of specific treatment.
- Individualization of therapy.

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7. REFERENCES

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.

[2] Cohen SM, Shirai T, Steineck G. Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol Suppl 2000:105-15.

[3] Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1513-30.

[4] Benhamou S, Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. Mutat Res 2000;462:149-58.

[5] Fryer AA, Jones PW. Interactions between detoxifying enzyme polymorphisms and susceptibility to cancer. IARC Sci Publ 1999:303-22.

[6] Brockmoller J, Cascorbi I, Kerb R, Sachse C, Roots I. Polymorphisms in xenobiotic conjugation and disease predisposition. Toxicol Lett 1998;102-103:173-83.

[7] Garcia-Closas M, Malats N, Real FX, Welch R, Kogevinas M, Chatterjee N, et al. Genetic variation in the nucleotide excision repair pathway and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:536-42.

[8] Gu J, Zhao H, Dinney CP, Zhu Y, Leibovici D, Bermejo CE, et al. Nucleotide excision repair gene polymorphisms and recurrence after treatment for superficial bladder cancer. Clin Cancer Res 2005;11:1408-15.

[9] Johne A, Roots I, Brockmoller J. A single nucleotide polymorphism in the human H-ras proto-oncogene determines the risk of urinary bladder cancer. Cancer Epidemiol Biomarkers Prev 2003;12:68-70.

[10] Johns LE, Houlston RS. Glutathione S-transferase mu1 (GSTM1) status and bladder cancer risk: a meta-analysis. Mutagenesis 2000;15:399-404.

[11] Knudsen KE, Diehl JA, Haiman CA, Knudsen ES. Cyclin D1: polymorphism, aberrant splicing and cancer risk. Oncogene 2006;25:1620-8.

[12] Sakano S, Wada T, Matsumoto H, Sugiyama S, Inoue R, Eguchi S, et al. Single nucleotide polymorphisms in DNA repair genes might be prognostic factors in muscle-invasive bladder cancer patients treated with chemoradiotherapy. Br J Cancer 2006.

[13] Wu X, Gu J, Grossman HB, Amos CI, Etzel C, Huang M, et al. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. Am J Hum Genet 2006;78:464-79.

[14] Wu X, Lin X, Dinney CP, Gu J, Grossman HB. Genetic polymorphism in bladder cancer. Front Biosci 2007;12:192-213.

[15] Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-6.

[16] Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106-30.

[17] Hall RR, Prout GR. Staging of bladder cancer: is the tumor, node, metastasis system adequate? Semin Oncol 1990;17:517-23.

[18] Mostofi FK DC, Sesterhnn IA: Histological typing of urinary bladder tumors WHO 1999.

[19] Malmstrom PU, Busch C, Norlen BJ. Recurrence, progression and survival in bladder cancer. A retrospective analysis of 232 patients with greater than or equal to 5-year follow-up. Scand J Urol Nephrol 1987;21:185-95.

[20] Larsson P, Wijkstrom H, Thorstenson A, Adolfsson J, Norming U, Wiklund P, et al. A population-based study of 538 patients with newly detected urinary bladder neoplasms followed during 5 years. Scand J Urol Nephrol 2003;37:195-201.

[21] Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. Int J Surg Pathol 2005;13:143-53.

[22] Knowles MA. Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese? Carcinogenesis 2006;27:361-73.

[23] Knowles MA. What we could do now: molecular pathology of bladder cancer. Mol Pathol 2001;54:215-21.

[24] Sylvester RJ, van der Meijden A, Witjes JA, Jakse G, Nonomura N, Cheng C, et al. High-grade Ta urothelial carcinoma and carcinoma in situ of the bladder. Urology 2005;66:90-107.

[25] Sengupta S, Blute ML. The management of superficial transitional cell carcinoma of the bladder. Urology 2006;67:48-54; discussion -5.

[26] Bolenz C, Cao Y, Arancibia MF, Trojan L, Alken P, Michel MS. Intravesical mitomycin C for superficial transitional cell carcinoma. Expert Rev Anticancer Ther 2006;6:1273-82.

[27] Nieder AM, Brausi M, Lamm D, O'Donnell M, Tomita K, Woo H, et al. Management of stage T1 tumors of the bladder: International Consensus Panel. Urology 2005;66:108-25.

[28] Lamm DL, McGee WR, Hale K. Bladder cancer: current optimal intravesical treatment. Urol Nurs 2005;25:323-6, 31-2.

[29] Schenkman E, Lamm DL. Superficial bladder cancer therapy. ScientificWorldJournal 2004;4 Suppl 1:387-99.

[30] Dinney CP. Therapy of invasive bladder cancer. Urology 2006;67:56-9; discussion 60-1.

[31] Logue J, McBain CA. Radiation therapy for muscle-invasive bladder cancer: treatment planning and delivery. Clin Oncol (R Coll Radiol) 2005;17:508-13.

[32] Steinmaus C, Moore L, Hopenhayn-Rich C, Biggs ML, Smith AH. Arsenic in drinking water and bladder cancer. Cancer Invest 2000;18:174-82.

[33] Tawfik HN. Carcinoma of the urinary bladder associated with schistosomiasis in Egypt: the possible causal relationship. Princess Takamatsu Symp 1987;18:197-209.

[34] Plna K, Hemminki K. Familial bladder cancer in the National Swedish Family Cancer Database. J Urol 2001;166:2129-33.

[35] Wolff EM, Liang G, Jones PA. Mechanisms of Disease: genetic and epigenetic alterations that drive bladder cancer. Nat Clin Pract Urol 2005;2:502-10.

[36] Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. Nat Rev Cancer 2005;5:713-25.

[37] Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. Genome Res 1998;8:1229-31.

[38] Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nat Genet 2003;33 Suppl:228-37.

[39] Brookes AJ. The essence of SNPs. Gene 1999;234:177-86.

[40] Imyanitov EN, Togo AV, Hanson KP. Searching for cancer-associated gene polymorphisms: promises and obstacles. Cancer Lett 2004;204:3-14.

[41] Friedberg EC. DNA damage and repair. Nature 2003;421:436-40.

[42] Sherr CJ. Cancer cell cycles. Science 1996;274:1672-7.

[43] Habuchi T. Common genetic polymorphisms and prognosis of sporadic cancers: prostate cancer as a model. Future Oncol 2006;2:233-45.

[44] Hemminki K, Thilly WG. Implications of results of molecular epidemiology on DNA adducts, their repair and mutations for mechanisms of human cancer. IARC Sci Publ 2004:217-35.

[45] Ryk C, Kumar R, Sanyal S, Verdier PJ, Hemminki K, Larsson P, et al. Influence of polymorphism in DNA repair and defence genes on p53 mutations in bladder tumours. Cancer Lett 2005.

[46] Ryk C, Berggren P, Kumar R, Hemminki K, Larsson P, Steineck G, et al. Influence of GSTM1, GSTT1, GSTP1 and NAT2 genotypes on the p53 mutational spectrum in bladder tumours. Int J Cancer 2005;113:761-8.

[47] Stern MC, Conway K, Li Y, Mistry K, Taylor JA. DNA repair gene polymorphisms and probability of p53 mutation in bladder cancer. Mol Carcinog 2006;45:715-9.

[48] Martone T, Vineis P, Malaveille C, Terracini B. Impact of polymorphisms in xeno(endo)biotic metabolism on pattern and frequency of p53 mutations in bladder cancer. Mutat Res 2000;462:303-9.

[49] Nagasubramanian R, Innocenti F, Ratain MJ. Pharmacogenetics in cancer treatment. Annu Rev Med 2003;54:437-52.

[50] Fojo T. Cancer, DNA repair mechanisms, and resistance to chemotherapy. J Natl Cancer Inst 2001;93:1434-6.

[51] Filippi AR, Franco P, Ricardi U. Is clinical radiosensitivity a complex genetically controlled event? Tumori 2006;92:87-91.

[52] de Chaisemartin L, Loriot MA. [Pharmacogenetics of anticancer drugs.]. Pathol Biol (Paris) 2005;53:116-24.

[53] Kari Hemminki JLB, Asta forsti. The balance between heritable and environmental aetiology of human disease. Nature Reviews 2006;7:958-65.

[54] Balmain A, Gray J, Ponder B. The genetics and genomics of cancer. Nat Genet 2003;33 Suppl:238-44.

[55] Hunter KW. Context-dependent cancer risk. Nat Genet 2006;38:864-5.

[56] Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, et al. Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. Genome Res 2001;11:143-51.

[57] Crawford DC, Nickerson DA. Definition and clinical importance of haplotypes. Annu Rev Med 2005;56:303-20.

[58] Brockmoller J, Cascorbi I, Henning S, Meisel C, Roots I. Molecular genetics of cancer susceptibility. Pharmacology 2000;61:212-27.

[59] Anzenbacher P, Anzenbacherova E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 2001;58:737-47.

[60] Rodriguez-Antona C, Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. Oncogene 2006;25:1679-91.

[61] Shimada T. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metab Pharmacokinet 2006;21:257-76.

[62] Liska DJ. The detoxification enzyme systems. Altern Med Rev 1998;3:187-98.

[63] Siegel D, McGuinness SM, Winski SL, Ross D. Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. Pharmacogenetics 1999;9:113-21.

[64] Ross D, Traver RD, Siegel D, Kuehl BL, Misra V, Rauth AM. A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity. Br J Cancer 1996;74:995-6.

[65] McCarver DG, Hines RN. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 2002;300:361-6.

[66] Ross RK, Jones PA, Yu MC. Bladder cancer epidemiology and pathogenesis. Semin Oncol 1996;23:536-45.

[67] Raunio H, Husgafvel-Pursiainen K, Anttila S, Hietanen E, Hirvonen A, Pelkonen O. Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility--a review. Gene 1995;159:113-21.

[68] Vineis P, Martone T. Molecular epidemiology of bladder cancer. Ann Ist Super Sanita 1996;32:21-7.

[69] Schlager JJ, Powis G. Cytosolic NAD(P)H:(quinone-acceptor)oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. Int J Cancer 1990;45:403-9.

[70] Joseph P, Jaiswal AK. NAD(P)H:quinone oxidoreductase1 (DT diaphorase) specifically prevents the formation of benzo[a]pyrene quinone-DNA adducts

generated by cytochrome P4501A1 and P450 reductase. Proc Natl Acad Sci U S A 1994;91:8413-7.

[71] Wu K, Knox R, Sun XZ, Joseph P, Jaiswal AK, Zhang D, et al. Catalytic properties of NAD(P)H:quinone oxidoreductase-2 (NQO2), a dihydronicotinamide riboside dependent oxidoreductase. Arch Biochem Biophys 1997;347:221-8.

[72] Benson AM. Conversion of 4-nitroquinoline 1-oxide (4NQO) to 4-hydroxyaminoquinoline 1-oxide by a dicumarol-resistant hepatic 4NQO nitroreductase in rats and mice. Biochem Pharmacol 1993;46:1217-21.

[73] Danson S, Ward TH, Butler J, Ranson M. DT-diaphorase: a target for new anticancer drugs. Cancer Treat Rev 2004;30:437-49.

[74] Traver RD, Horikoshi T, Danenberg KD, Stadlbauer TH, Danenberg PV, Ross D, et al. NAD(P)H:quinone oxidoreductase gene expression in human colon carcinoma cells: characterization of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. Cancer Res 1992;52:797-802.

[75] Chao C, Zhang ZF, Berthiller J, Boffetta P, Hashibe M. NAD(P)H:quinone oxidoreductase 1 (NQO1) Pro187Ser polymorphism and the risk of lung, bladder, and colorectal cancers: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:979-87.

[76] Hung RJ, Boffetta P, Brennan P, Malaveille C, Gelatti U, Placidi D, et al. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. Carcinogenesis 2004;25:973-8.

[77] Schulz WA, Krummeck A, Rosinger I, Eickelmann P, Neuhaus C, Ebert T, et al. Increased frequency of a null-allele for NAD(P)H: quinone oxidoreductase in patients with urological malignancies. Pharmacogenetics 1997;7:235-9.

[78] Gaedigk A, Tyndale RF, Jurima-Romet M, Sellers EM, Grant DM, Leeder JS. NAD(P)H:quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations. Pharmacogenetics 1998;8:305-13.

[79] Eguchi-Ishimae M, Eguchi M, Ishii E, Knight D, Sadakane Y, Isoyama K, et al. The association of a distinctive allele of NAD(P)H:quinone oxidoreductase with pediatric acute lymphoblastic leukemias with MLL fusion genes in Japan. Haematologica 2005;90:1511-5.

[80] Moore LE, Wiencke JK, Bates MN, Zheng S, Rey OA, Smith AH. Investigation of genetic polymorphisms and smoking in a bladder cancer case-control study in Argentina. Cancer Lett 2004;211:199-207.

[81] Park SJ, Zhao H, Spitz MR, Grossman HB, Wu X. An association between NQO1 genetic polymorphism and risk of bladder cancer. Mutat Res 2003;536:131-7.

[82] Choi JY, Lee KM, Cho SH, Kim SW, Choi HY, Lee SY, et al. CYP2E1 and NQO1 genotypes, smoking and bladder cancer. Pharmacogenetics 2003;13:349-55.

[83] Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005;366:649-59.

[84] Gu J, Liang D, Wang Y, Lu C, Wu X. Effects of N-acetyl transferase 1 and 2 polymorphisms on bladder cancer risk in Caucasians. Mutat Res 2005;581:97-104.

[85] Hein DW. N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. Oncogene 2006;25:1649-58.

[86] Cascorbi I, Roots I, Brockmoller J. Association of NAT1 and NAT2 polymorphisms to urinary bladder cancer: significantly reduced risk in subjects with NAT1*10. Cancer Res 2001;61:5051-6.

[87] Cao W, Cai L, Rao JY, Pantuck A, Lu ML, Dalbagni G, et al. Tobacco smoking, GSTP1 polymorphism, and bladder carcinoma. Cancer 2005;104:2400-8.

[88] Srivastava DS, Mishra DK, Mandhani A, Mittal B, Kumar A, Mittal RD. Association of genetic polymorphism of glutathione S-transferase M1, T1, P1 and susceptibility to bladder cancer. Eur Urol 2005;48:339-44.

[89] Saad AA, O'Connor PJ, Mostafa MH, Metwalli NE, Cooper DP, Povey AC, et al. Glutathione S-transferase M1, T1 and P1 polymorphisms and bladder cancer risk in Egyptians. Int J Biol Markers 2005;20:69-72.

[90] Mittal RD, Srivastava DS, A M, B M. Genetic polymorphism of drug metabolizing enzymes (CYP2E1, GSTP1) and susceptibility to bladder cancer in North India. Asian Pac J Cancer Prev 2005;6:6-9.

[91] Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, et al. Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. Urology 2005;65:70-5.

[92] Sobti RC, Al-Badran AI, Sharma S, Sharma SK, Krishan A, Mohan H. Genetic polymorphisms of CYP2D6, GSTM1, and GSTT1 genes and bladder cancer risk in North India. Cancer Genet Cytogenet 2005;156:68-73.

[93] Hung RJ BP, Brennan P, Malaveille C, Hautefeuille A, Donato F, Gelatti U, Spaliviero M, Placidi D, Carta A, Scotto di Carlo A, Porru S. GST, NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. Int J Cancer 2004 Jul 1;110:598-604.

[94] Srivastava DS, Kumar A, Mittal B, Mittal RD. Polymorphism of GSTM1 and GSTT1 genes in bladder cancer: a study from North India. Arch Toxicol 2004;78:430-4.

[95] Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, et al. Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 2004;25:729-34.

[96] Karagas MR, Park S, Warren A, Hamilton J, Nelson HH, Mott LA, et al. Gender, smoking, glutathione-S-transferase variants and bladder cancer incidence: a population-based study. Cancer Lett 2005;219:63-9.

[97] Lin J, Spitz MR, Wang Y, Schabath MB, Gorlov IP, Hernandez LM, et al. Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: a case-control study. Carcinogenesis 2004;25:1639-47.

[98] Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, et al. Metabolic activation and deactivation of arylamine carcinogens by recombinant human

NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 1993;14:1633-8.

[99] Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 1993;85:1159-64.

[100] Hickman D, Risch A, Camilleri JP, Sim E. Genotyping human polymorphic arylamine N-acetyltransferase: identification of new slow allotypic variants. Pharmacogenetics 1992;2:217-26.

[101] Marcus PM, Vineis P, Rothman N. NAT2 slow acetylation and bladder cancer risk: a meta-analysis of 22 case-control studies conducted in the general population. Pharmacogenetics 2000;10:115-22.

[102] Hamasaki T, Inatomi H, Katoh T, Aono H, Ikuyama T, Muratani T, et al. N-acetyltransferase-2 gene polymorphism as a possible biomarker for prostate cancer in Japanese men. Int J Urol 2003;10:167-73.

[103] Bolt HM, Thier R. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab 2006;7:613-28.

[104] Lecomte T, Landi B, Beaune P, Laurent-Puig P, Loriot MA. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. Clin Cancer Res 2006;12:3050-6.

[105] McIlwain CC, Townsend DM, Tew KD. Glutathione S-transferase polymorphisms: cancer incidence and therapy. Oncogene 2006;25:1639-48.

[106] Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem 1997;272:10004-12.

[107] Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 1997;18:641-4.

[108] Harris MJ, Coggan M, Langton L, Wilson SR, Board PG. Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. Pharmacogenetics 1998;8:27-31.

[109] Martinez C, Martin F, Fernandez JM, Garcia-Martin E, Sastre J, Diaz-Rubio M, et al. Glutathione S-transferases mu 1, theta 1, pi 1, alpha 1 and mu 3 genetic polymorphisms and the risk of colorectal and gastric cancers in humans. Pharmacogenomics 2006;7:711-8.

[110] Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177-82.

[111] Smith CM, Kelsey KT, Wiencke JK, Leyden K, Levin S, Christiani DC. Inherited glutathione-S-transferase deficiency is a risk factor for pulmonary asbestosis. Cancer Epidemiol Biomarkers Prev 1994;3:471-7.

[112] Mohr LC, Rodgers JK, Silvestri GA. Glutathione S-transferase M1 polymorphism and the risk of lung cancer. Anticancer Res 2003;23:2111-24.

[113] Warnecke PM, Bestor TH. Cytosine methylation and human cancer. Curr Opin Oncol 2000;12:68-73.

[114] Kim YI. 5,10-Methylenetetrahydrofolate reductase polymorphisms and pharmacogenetics: a new role of single nucleotide polymorphisms in the folate metabolic pathway in human health and disease. Nutr Rev 2005;63:398-407.

[115] Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098-102.

[116] Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A 1999;96:12810-5.

[117] Shen H, Xu Y, Zheng Y, Qian Y, Yu R, Qin Y, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: a case-control study. Int J Cancer 2001;95:332-6.

[118] Ziegler RG, Weinstein SJ, Fears TR. Nutritional and genetic inefficiencies in one-carbon metabolism and cervical cancer risk. J Nutr 2002;132:2345S-9S.

[119] Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 2002;50:520-4.

[120] Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290-5.

[121] Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol 2003;157:571-82.

[122] Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159:423-43.

[123] Hess MT, Schwitter U, Petretta M, Giese B, Naegeli H. Bipartite substrate discrimination by human nucleotide excision repair. Proc Natl Acad Sci U S A 1997;94:6664-9.

[124] Batty DP, Wood RD. Damage recognition in nucleotide excision repair of DNA. Gene 2000;241:193-204.

[125] Friedberg EC. How nucleotide excision repair protects against cancer. Nat Rev Cancer 2001;1:22-33.

[126] Memisoglu A, Samson L. Base excision repair in yeast and mammals. Mutat Res 2000;451:39-51.

[127] Matsumoto Y, Kim K. Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. Science 1995;269:699-702.

[128] Matsumoto Y, Kim K, Bogenhagen DF. Proliferating cell nuclear antigen-dependent abasic site repair in Xenopus laevis oocytes: an alternative pathway of base excision DNA repair. Mol Cell Biol 1994;14:6187-97.

[129] Frosina G, Fortini P, Rossi O, Carrozzino F, Raspaglio G, Cox LS, et al. Two pathways for base excision repair in mammalian cells. J Biol Chem 1996;271:9573-8.

[130] Hung RJ, Hall J, Brennan P, Boffetta P. Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol 2005;162:925-42.

[131] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366-74.

[132] Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis 2002;23:687-96.

[133] Sonoda E, Takata M, Yamashita YM, Morrison C, Takeda S. Homologous DNA recombination in vertebrate cells. Proc Natl Acad Sci U S A 2001;98:8388-94.

[134] Featherstone C, Jackson SP. DNA double-strand break repair. Curr Biol 1999;9:R759-61.

[135] Dasika GK, Lin SC, Zhao S, Sung P, Tomkinson A, Lee EY. DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. Oncogene 1999;18:7883-99.

[136] Genschel J, Littman SJ, Drummond JT, Modrich P. Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. J Biol Chem 1998;273:19895-901.

[137] Gradia S, Subramanian D, Wilson T, Acharya S, Makhov A, Griffith J, et al. hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA. Mol Cell 1999;3:255-61.

[138] Blackwell LJ, Martik D, Bjornson KP, Bjornson ES, Modrich P. Nucleotide-promoted release of hMutSalpha from heteroduplex DNA is consistent with an ATP-dependent translocation mechanism. J Biol Chem 1998;273:32055-62.

[139] Buermeyer AB, Deschenes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. Annu Rev Genet 1999;33:533-64.

[140] Khan SG, Muniz-Medina V, Shahlavi T, Baker CC, Inui H, Ueda T, et al. The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. Nucleic Acids Res 2002;30:3624-31.

[141] Yokoi M, Masutani C, Maekawa T, Sugasawa K, Ohkuma Y, Hanaoka F. The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA. J Biol Chem 2000;275:9870-5.

[142] D'Errico M, Parlanti E, Teson M, de Jesus BM, Degan P, Calcagnile A, et al. New functions of XPC in the protection of human skin cells from oxidative damage. Embo J 2006;25:4305-15.

[143] Khan SG, Metter EJ, Tarone RE, Bohr VA, Grossman L, Hedayati M, et al. A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. Carcinogenesis 2000;21:1821-5.

[144] Chavanne F, Broughton BC, Pietra D, Nardo T, Browitt A, Lehmann AR, et al. Mutations in the XPC gene in families with xeroderma pigmentosum and consequences at the cell, protein, and transcript levels. Cancer Res 2000;60:1974-82.

[145] Lee GY, Jang JS, Lee SY, Jeon HS, Kim KM, Choi JE, et al. XPC polymorphisms and lung cancer risk. Int J Cancer 2005;115:807-13.

[146] Shen H, Sturgis EM, Khan SG, Qiao Y, Shahlavi T, Eicher SA, et al. An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Res 2001;61:3321-5.

[147] Blankenburg S, Konig IR, Moessner R, Laspe P, Thoms KM, Krueger U, et al. Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case-control study. Carcinogenesis 2005;26:1085-90.

[148] Hu Z, Wang Y, Wang X, Liang G, Miao X, Xu Y, et al. DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. Int J Cancer 2005;115:478-83.

[149] Huang WY, Berndt SI, Kang D, Chatterjee N, Chanock SJ, Yeager M, et al. Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: XPC polymorphisms modify smoking-related risk. Cancer Epidemiol Biomarkers Prev 2006;15:306-11.

[150] Weiss JM, Weiss NS, Ulrich CM, Doherty JA, Voigt LF, Chen C. Interindividual variation in nucleotide excision repair genes and risk of endometrial cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2524-30.

[151] Sak SC, Barrett JH, Paul AB, Bishop DT, Kiltie AE. Comprehensive Analysis of 22 XPC Polymorphisms and Bladder Cancer Risk. Cancer Epidemiol Biomarkers Prev 2006;15:2537-41.

[152] Zhu Y, Lai M, Yang H, Lin J, Huang M, Grossman HB, et al. Genotypes, haplotypes, and diplotypes of XPC and risk of bladder cancer. Carcinogenesis 2006.

[153] Coin F, Marinoni JC, Rodolfo C, Fribourg S, Pedrini AM, Egly JM. Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. Nat Genet 1998;20:184-8.

[154] Reardon JT, Ge H, Gibbs E, Sancar A, Hurwitz J, Pan ZQ. Isolation and characterization of two human transcription factor IIH (TFIIH)-related complexes: ERCC2/CAK and TFIIH. Proc Natl Acad Sci U S A 1996;93:6482-7.

[155] Taylor EM, Broughton BC, Botta E, Stefanini M, Sarasin A, Jaspers NG, et al. Xeroderma pigmentosum and trichothiodystrophy are associated with different mutations in the XPD (ERCC2) repair/transcription gene. Proc Natl Acad Sci U S A 1997;94:8658-63.

[156] Seker H, Butkiewicz D, Bowman ED, Rusin M, Hedayati M, Grossman L, et al. Functional significance of XPD polymorphic variants: attenuated apoptosis in

human lymphoblastoid cells with the XPD 312 Asp/Asp genotype. Cancer Res 2001;61:7430-4.

[157] Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000;21:551-5.

[158] Debniak T, Scott RJ, Huzarski T, Byrski T, Masojc B, van de Wetering T, et al. XPD common variants and their association with melanoma and breast cancer risk. Breast Cancer Res Treat 2006;98:209-15.

[159] Schabath MB, Delclos GL, Grossman HB, Wang Y, Lerner SP, Chamberlain RM, et al. Polymorphisms in XPD exons 10 and 23 and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:878-84.

[160] Butkiewicz D, Rusin M, Enewold L, Shields PG, Chorazy M, Harris CC. Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis 2001;22:593-7.

[161] Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 2000;21:965-71.

[162] Vodicka P, Kumar R, Stetina R, Sanyal S, Soucek P, Haufroid V, et al. Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. Carcinogenesis 2004;25:757-63.

[163] Angelini S, Kumar R, Carbone F, Maffei F, Forti GC, Violante FS, et al. Micronuclei in humans induced by exposure to low level of ionizing radiation: influence of polymorphisms in DNA repair genes. Mutat Res 2005;570:105-17.

[164] Benhamou S, Sarasin A. ERCC2 /XPD gene polymorphisms and lung cancer: a HuGE review. Am J Epidemiol 2005;161:1-14.

[165] Mu D, Hsu DS, Sancar A. Reaction mechanism of human DNA repair excision nuclease. J Biol Chem 1996;271:8285-94.

[166] O'Donovan A, Davies AA, Moggs JG, West SC, Wood RD. XPG endonuclease makes the 3' incision in human DNA nucleotide excision repair. Nature 1994;371:432-5.

[167] Mu D, Sancar A. Model for XPC-independent transcription-coupled repair of pyrimidine dimers in humans. J Biol Chem 1997;272:7570-3.

[168] Gary R, Ludwig DL, Cornelius HL, MacInnes MA, Park MS. The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA-binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. J Biol Chem 1997;272:24522-9.

[169] Shivji KK, Kenny MK, Wood RD. Proliferating cell nuclear antigen is required for DNA excision repair. Cell 1992;69:367-74.

[170] Neumann AS, Sturgis EM, Wei Q. Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. Mol Carcinog 2005;42:65-92.

[171] Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, et al. Polymorphism of Xeroderma Pigmentosum group G and the risk of lung cancer

and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer 2006;118:714-20.

[172] Kumar R, Hoglund L, Zhao C, Forsti A, Snellman E, Hemminki K. Single nucleotide polymorphisms in the XPG gene: determination of role in DNA repair and breast cancer risk. Int J Cancer 2003;103:671-5.

[173] Lockett KL, Snowhite IV, Hu JJ. Nucleotide-excision repair and prostate cancer risk. Cancer Lett 2005;220:125-35.

[174] Kristensen VN, Edvardsen H, Tsalenko A, Nordgard SH, Sorlie T, Sharan R, et al. Genetic variation in putative regulatory loci controlling gene expression in breast cancer. Proc Natl Acad Sci U S A 2006;103:7735-40.

[175] Qiao Y, Spitz MR, Shen H, Guo Z, Shete S, Hedayati M, et al. Modulation of repair of ultraviolet damage in the host-cell reactivation assay by polymorphic XPC and XPD/ERCC2 genotypes. Carcinogenesis 2002;23:295-9.

[176] Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001;61:1354-7.

[177] Stern MC, Johnson LR, Bell DA, Taylor JA. XPD codon 751 polymorphism, metabolism genes, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1004-11.

[178] Sakano S, Kumar R, Larsson P, Onelov E, Adolfsson J, Steineck G, et al. A single-nucleotide polymorphism in the XPG gene, and tumour stage, grade, and clinical course in patients with nonmuscle-invasive neoplasms of the urinary bladder. BJU Int 2006;97:847-51.

[179] Au WW, Salama SA, Sierra-Torres CH. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ Health Perspect 2003;111:1843-50.

[180] Shen M, Hung RJ, Brennan P, Malaveille C, Donato F, Placidi D, et al. Polymorphisms of the DNA repair genes XRCC1, XRCC3, XPD, interaction with environmental exposures, and bladder cancer risk in a case-control study in northern Italy. Cancer Epidemiol Biomarkers Prev 2003;12:1234-40.

[181] Stern MC, Umbach DM, van Gils CH, Lunn RM, Taylor JA. DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:125-31.

[182] Kelsey KT, Park S, Nelson HH, Karagas MR. A population-based case-control study of the XRCC1 Arg399Gln polymorphism and susceptibility to bladder cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1337-41.

[183] Sak SC, Harnden P, Johnston CF, Paul AB, Kiltie AE. APE1 and XRCC1 protein expression levels predict cancer-specific survival following radical radiotherapy in bladder cancer. Clin Cancer Res 2005;11:6205-11.

[184] Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, et al. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 1998;16:3219-25.

[185] Matullo G, Palli D, Peluso M, Guarrera S, Carturan S, Celentano E, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001;22:1437-45.

[186] Stern MC, Umbach DM, Lunn RM, Taylor JA. DNA repair gene XRCC3 codon 241 polymorphism, its interaction with smoking and XRCC1 polymorphisms, and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:939-43.

[187] Kawakami T, Shiina H, Igawa M, Deguchi M, Nakajima K, Ogishima T, et al. Inactivation of the hMSH3 mismatch repair gene in bladder cancer. Biochem Biophys Res Commun 2004;325:934-42.

[188] Sanyal S dVP, Steineck G, Larsson P, Onelöv E, Hemminki K, Kumar R. Polymorphisms in XPD, XPC and the risk of death in patients with urinary bladder neoplasms. Acta Oncologica 2006.

[189] Lindahl T, Wood RD. Quality control by DNA repair. Science 1999;286:1897-905.

[190] Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, Frosina G. Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J Biol Chem 1997;272:23970-5.

[191] Fan J, Otterlei M, Wong HK, Tomkinson AE, Wilson DM, 3rd. XRCC1 co-localizes and physically interacts with PCNA. Nucleic Acids Res 2004;32:2193-201.

[192] Taylor RM, Thistlethwaite A, Caldecott KW. Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair. Mol Cell Biol 2002;22:2556-63.

[193] Hu Z, Ma H, Chen F, Wei Q, Shen H. XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. Cancer Epidemiol Biomarkers Prev 2005;14:1810-8.

[194] Shinmura K, Yokota J. The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. Antioxid Redox Signal 2001;3:597-609.

[195] Weiss JM, Goode EL, Ladiges WC, Ulrich CM. Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. Mol Carcinog 2005;42:127-41.

[196] Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev 1999;13:2633-8.

[197] Brenneman MA, Weiss AE, Nickoloff JA, Chen DJ. XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. Mutat Res 2000;459:89-97.

[198] Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, et al. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol Cell 1998;1:783-93.

[199] Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, et al. Xrcc3 is required for assembly of Rad51 complexes in vivo. J Biol Chem 1998;273:21482-8.

[200] Liu Y, Masson JY, Shah R, O'Regan P, West SC. RAD51C is required for Holliday junction processing in mammalian cells. Science 2004;303:243-6.

[201] Manuguerra M, Saletta F, Karagas MR, Berwick M, Veglia F, Vineis P, et al. XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. Am J Epidemiol 2006;164:297-302.

[202] Thirumaran RK, Bermejo JL, Rudnai P, Gurzau E, Koppova K, Goessler W, et al. Single nucleotide polymorphisms in DNA repair genes and basal cell carcinoma of skin. Carcinogenesis 2006;27:1676-81.

[203] Winsey SL, Haldar NA, Marsh HP, Bunce M, Marshall SE, Harris AL, et al. A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res 2000;60:5612-6.

[204] Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A, Lipscombe JM, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 2002;11:1399-407.

[205] Shen H, Sturgis EM, Dahlstrom KR, Zheng Y, Spitz MR, Wei Q. A variant of the DNA repair gene XRCC3 and risk of squamous cell carcinoma of the head and neck: a case-control analysis. Int J Cancer 2002;99:869-72.

[206] Arthur LM, Gustausson K, Hopfner KP, Carson CT, Stracker TH, Karcher A, et al. Structural and functional analysis of Mre11-3. Nucleic Acids Res 2004;32:1886-93.

[207] Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, et al. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res 2003;63:2440-6.

[208] Zhang Y, Zhou J, Lim CU. The role of NBS1 in DNA double strand break repair, telomere stability, and cell cycle checkpoint control. Cell Res 2006;16:45-54.

[209] Weemaes CM, Hustinx TW, Scheres JM, van Munster PJ, Bakkeren JA, Taalman RD. A new chromosomal instability disorder: the Nijmegen breakage syndrome. Acta Paediatr Scand 1981;70:557-64.

[210] Kobayashi J, Antoccia A, Tauchi H, Matsuura S, Komatsu K. NBS1 and its functional role in the DNA damage response. DNA Repair (Amst) 2004;3:855-61.

[211] Lu J, Wei Q, Bondy ML, Li D, Brewster A, Shete S, et al. Polymorphisms and haplotypes of the NBS1 gene are associated with risk of sporadic breast cancer in non-Hispanic white women <= 55 years. Carcinogenesis 2006.

[212] Ryk C, Kumar R, Thirumaran RK, Hou SM. Polymorphisms in the DNA repair genes XRCC1, APEX1, XRCC3 and NBS1, and the risk for lung cancer in never- and ever-smokers. Lung Cancer 2006.

[213] Medina PP, Ahrendt SA, Pollan M, Fernandez P, Sidransky D, Sanchez-Cespedes M. Screening of homologous recombination gene polymorphisms in lung cancer patients reveals an association of the NBS1-185Gln variant and p53 gene mutations. Cancer Epidemiol Biomarkers Prev 2003;12:699-704.

[214] Huang J, Kuismanen SA, Liu T, Chadwick RB, Johnson CK, Stevens MW, et al. MSH6 and MSH3 are rarely involved in genetic predisposition to nonpolypotic colon cancer. Cancer Res 2001;61:1619-23.

[215] Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D. High resolution deletion mapping reveals frequent allelic losses at the DNA

mismatch repair loci hMLH1 and hMSH3 in non-small cell lung cancer. Int J Cancer 1998;77:173-80.

[216] Nakajima E, Orimo H, Ikejima M, Shimada T. Nine-bp repeat polymorphism in exon 1 of the hMSH3 gene. Jpn J Hum Genet 1995;40:343-5.

[217] Orimo H, Nakajima E, Ikejima M, Emi M, Shimada T. Frameshift mutations and a length polymorphism in the hMSH3 gene and the spectrum of microsatellite instability in sporadic colon cancer. Jpn J Cancer Res 1999;90:1310-5.

[218] Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 1995;268:1909-12.

[219] Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, D'Arrigo A, et al. GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. Science 1995;268:1912-4.

[220] Peltomaki P. Lynch syndrome genes. Fam Cancer 2005;4:227-32.

[221] Song H, Ramus SJ, Quaye L, Dicioccio RA, Tyrer J, Lomas E, et al. Common variants in mismatch repair genes and risk of invasive ovarian cancer. Carcinogenesis 2006.

[222] Prescott DM. The cell cycle and the control of cellular reproduction. Adv Genet 1976;18:99-177.

[223] Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science 1989;246:629-34.

[224] Barbacid M. ras genes. Annu Rev Biochem 1987;56:779-827.

[225] Lowy DR, Willumsen BM. Function and regulation of ras. Annu Rev Biochem 1993;62:851-91.

[226] Hunter T. Oncoprotein networks. Cell 1997;88:333-46.

[227] Cohen JB, Broz SD, Levinson AD. Expression of the H-ras proto-oncogene is controlled by alternative splicing. Cell 1989;58:461-72.

[228] Huang MY, Cohen JB. The alternative H-ras protein p19 displays properties of a negative regulator of p21Ras. Oncol Res 1997;9:611-21.

[229] Guil S, de La Iglesia N, Fernandez-Larrea J, Cifuentes D, Ferrer JC, Guinovart JJ, et al. Alternative splicing of the human proto-oncogene c-H-ras renders a new Ras family protein that trafficks to cytoplasm and nucleus. Cancer Res 2003;63:5178-87.

[230] Bos JL. The ras gene family and human carcinogenesis. Mutat Res 1988;195:255-71.

[231] Bos JL. ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682-9.

[232] Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature 1982;300:762-5.

[233] Reddy EP. Nucleotide sequence analysis of the T24 human bladder carcinoma oncogene. Science 1983;220:1061-3.

[234] Sherr CJ. D-type cyclins. Trends Biochem Sci 1995;20:187-90.

[235] Cobrinik D, Dowdy SF, Hinds PW, Mittnacht S, Weinberg RA. The retinoblastoma protein and the regulation of cell cycling. Trends Biochem Sci 1992;17:312-5.

[236] Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 1999;98:859-69.

[237] Ruas M, Peters G. The p16INK4a/CDKN2A tumor suppressor and its relatives. Biochim Biophys Acta 1998;1378:F115-77.

[238] Donnellan R, Chetty R. Cyclin D1 and human neoplasia. Mol Pathol 1998;51:1-7.

[239] Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, et al. Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 1999;5:1966-75.

[240] Wagner U, Suess K, Luginbuhl T, Schmid U, Ackermann D, Zellweger T, et al. Cyclin D1 overexpression lacks prognostic significance in superficial urinary bladder cancer. J Pathol 1999;188:44-50.

[241] Lee CC, Yamamoto S, Morimura K, Wanibuchi H, Nishisaka N, Ikemoto S, et al. Significance of cyclin D1 overexpression in transitional cell carcinomas of the urinary bladder and its correlation with histopathologic features. Cancer 1997;79:780-9.

[242] Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995;11:1005-11.

[243] Sawa H, Ohshima TA, Ukita H, Murakami H, Chiba Y, Kamada H, et al. Alternatively spliced forms of cyclin D1 modulate entry into the cell cycle in an inverse manner. Oncogene 1998;16:1701-12.

[244] Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, Kakehi Y, et al. Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 2002;23:257-64.

[245] Ito M, Habuchi T, Watanabe J, Higashi S, Nishiyama H, Wang L, et al. Polymorphism within the cyclin D1 gene is associated with an increased risk of carcinoma in situ in patients with superficial bladder cancer. Urology 2004;64:74-8.

[246] Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273:1516-7.

[247] Cortessis VK, Siegmund K, Xue S, Ross RK, Yu MC. A case-control study of cyclin D1 CCND1 870A-->G polymorphism and bladder cancer. Carcinogenesis 2003;24:1645-50.

[248] D Coggon GR, DJP Barker. Epidemiology for the uninitiated, Fourth Edition. British Medical Journal home 1997.

[249] Cardon LR, Bell JI. Association study designs for complex diseases. Nat Rev Genet 2001;2:91-9.

[250] Steineck G, Hunt H, Adolfsson J. A hierarchical step-model for causation of bias-evaluating cancer treatment with epidemiological methods. Acta Oncol 2006;45:421-9.

[251] Vogel U, Overvad K, Wallin H, Tjonneland A, Nexo BA, Raaschou-Nielsen O. Combinations of polymorphisms in XPD, XPC and XPA in relation to risk of lung cancer. Cancer Lett 2005;222:67-74.

[252] Bernstein C, Bernstein H, Payne CM, Garewal H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res 2002;511:145-78.

[253] Stout GJ, Oosten MV, Acherrat FZ, Wit JD, Vermeij WP, Mullenders LH, et al. Selective DNA damage responses in murine Xpa(-/-), Xpc(-/-) and Csb(-/-) keratinocyte cultures. DNA Repair (Amst) 2005.

[254] Perneger TV. What's wrong with Bonferroni adjustments. Bmj 1998;316:1236-8.