THE ASSOCIATION OF DNA REPAIR GENE POLYMORPHISMS WITH CARCINOMA PROSTATE

Dissertation submitted to

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DECLARATION

I solemnly declare that this dissertation titled **THE ASSOCIATION OF DNA REPAIR GENE POLYMORPHISMS WITH CARCINOMA PROSTATE** was prepared by me in the Department of Urology, Government General Hospital, Chennai under the guidance and supervision of **Prof. R. Jeyaraman, M.Ch .,** Professor & Head of the Department, Department of urology, Government General Hospital, Chennai. This dissertation is submitted to the Tamil Nadu Dr. MGR Medical University, Chennai in partial fulfillment of the university requirements for the award of the degree of M.Ch. Urology.

Place: Chennai

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CERTIFICATE

This is to certify that the dissertation titled "**THE ASSOCIATION OF DNA REPAIR GENE POLYMORPHISMS WITH CARCINOMA PROSTATE**" submitted by **Dr.I.C.IYAL AMUTHAN** appearing for **M.Ch. (Urology)** degree examination in August 2011, is a bonafide record of work done by him under the guidance and supervision of the Professor and HOD , Department of Urology in partial fulfillment of requirements of the TamilNadu Dr.M.G.R.Medical University, Chennai. This dissertation is forwarded to the Tamil Nadu Dr.M.G.R.Medical University, Chennai.

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INTRODUCTION

Prostate cancer is the most common noncutaneous malignancy in men in the United States. In India, the incidence is relatively less .Risk of disease varies most prominently with age, ethnicity, family history, and diet . A strong family history indicative of a highly penetrant prostate cancer gene is believed to account for only 5–10% of prostate cancers, whereas a larger percentage of prostate cancers may be due to common polymorphisms in genes giving rise to a low penetrance risk of disease[1-3.] Malignant transformation of prostate cells is accompanied by somatic genomic changes, including deletions, amplifications, and point mutations [4,5.] *In vitro* studies of human prostate tissue have demonstrated that DNA adducts form in prostate tissue after exposure to environmental toxins [6,7.] Moreover, intake of antioxidants via the diet or as supplements may decrease prostate cancer risk through the inactivation of reactive oxygen species, thereby protecting the DNA from oxidative damage [8.]

This evidence suggests that DNA repair capacity may play an important role in prostate carcinogenesis, but little is known about what direct effect DNA repair capacity has on prostate cancer risk.

XRCC1 is involved in DNA repair in the base excision repair pathway and appears to play a scaffolding role in bringing together a complex of DNA repair proteins, including poly (ADP-ribose) polymerase (PARP), DNA ligase 3 (LIG3), and DNA polymerase [9 –11.] Codons 194 and 399 contain polymorphisms that result in amino acid substitutions within evolutionarily conserved regions [12, 13.] Several studies have linked *XRCC1* polymorphisms with biomarkers of DNA damage, including aflatoxin B1-DNA adducts and glycophorin A variants in erythrocytes [14], polyphenol-DNA adducts[15], and DNA repair capacity in lymphocyte[16].

The *XPD* gene codes for a DNA helicase involved in transcription and nucleotide excision repair. Mutations in the *XPD* gene can completely prevent DNA opening and dual incision, steps that lead to the repair of DNA adducts[17.] The DNA repair function of XPD is critical to reparation of genetic damage from tobacco and other carcinogens [18]. Several common single bp substitution polymorphisms in the *XPD* gene have been identified.

AIM AND OBJECTIVES

The aim of the study is to determine the risk attributed by polymorphisms in genes regulating the DNA Repair pathway with reference to the Xray repair Cross complementary 1 gene (XRCC1) and Xeroderma pigmentosum group D gene (XPD) with Carcinoma Prostate.

The study also aims to perform a stratified analysis of the genotypes with Age, Gleason sum and Serum PSA levels of Prostate Cancer patients.

REVIEW OF LITERATURE

EPIDEMIOLOGY

Worldwide Incidence and Mortality

Prostate cancer is the fourth most common male malignant neoplasm worldwide. Its incidence varies widely between countries and ethnic populations, and disease rates differ by more than 100-fold between populations. The lowest yearly incidence rates occur in Asia (1.9 cases per 100,000 in Tianjin, China) and the highest in North America and Scandinavia, especially in African Americans (272 cases per 100,000)[19]. As in the United States, prostate cancer incidence has increased in many countries since the early 1990s. Although much of the increase can be correlated with the introduction of PSA screening, some of the increase predates screening[20]. Mortality also varies widely among countries, being highest in Sweden (23 per 100,000 per year) and lowest in Asia (<5 per 100,000 per year in Singapore, Japan, and China). Mortality rates increased slowly for most countries between 1985 and 1995 .

There are multiple complex causes for the worldwide and ethnic variations in prostate cancer incidence. Access to and quality of health

care, accuracy of cancer registries, and penetrance of PSA screening affect how rates of disease are reported. Before reliable data were available from African countries, rates of prostate cancer in Africa were thought to be much the same as those in Asia.

However, in Uganda and Nigeria, prostate cancer is common, and it is the most common cancer in men in Nigeria. Environment also plays an important role in modulating prostate cancer risk around the world. Japanese and Chinese men in the United States have a higher risk for development of prostate cancer and dying of it than do their relatives in Japan and China [21,22]. Likewise, prostate cancer incidence and mortality have increased in Japan as the country has become more westernized [23]. However, Asian Americans have a lower prostate cancer incidence than white or African American men do, indicating that genetics still plays a role in determining prostate cancer predisposition.

Age at Diagnosis

Prostate cancer is rarely diagnosed in men younger than 50 years, accounting for less than 0.1% of all patients. Peak incidence occurs between the ages of 70 and 74 years, with 85% diagnosed after the age of 65 years[24]. At 85 years of age, the cumulative risk of clinically diagnosed prostate cancer ranges from 0.5% to 20% worldwide, despite autopsy

evidence of microscopic lesions in approximately 30% of men in the fourth decade, 50% of men in the sixth decade, and more than 75% of men older than 85 years[25]. PSA-based screening has induced an important age migration effect; the incidence of prostate cancer in men 50 to 59 years has increased by 50% between 1989 and 1992[26] , with important implications for deciding on the need for, type of, and complications after therapy.

RISK FACTORS

Familial and Genetic Influences

Ample epidemiologic evidence suggests that prostate cancer has both a familial and genetic component. The first reports of a familial clustering were published in the mid- 20th century and suggested that the risk for development of prostate cancer was higher in those with an affected first-degree relative [27] .

Subsequent case-control and cohort studies have confirmed this observation[28]. Twin studies have also suggested a genetic component, with higher rates of concordance for monozygotic than for dizygotic brothers[29,30] Relative risk increases according to the number of affected family members, their degree of relatedness, and the age at which they were affected.

Familial and Genetic Influences

Relative risk increases according to the number of affected family members, their degree of relatedness, and the age at which they were affected

Family History	Relative Risk	Absolute Risk (%)
None	1	8
Father or brother	2	15
Father or brother affected < 60 years	3	20
Father and brother	4	30
Hereditary prostate cancer	5	35-45

SUSCEPTIBLE GENES

Evidence for major prostate cancer susceptibility genes that segregate in families has been obtained from several complex segregation analyses, with the majority supporting a dominant and the remainder supporting a recessive or X-linked mode of inheritance[31].

At least eight candidate prostate cancer susceptibility genes have been reported, including RNase L/*HPC1*[32], *ELAC2/HPC2*[33], SR- A/*MSR1*[34], *CHEK2*[35], *BRCA2*[36], *PON1*[37], *OGG1*[38], and *MIC1*[39]. Individually, these genes are likely to account for only a small fraction of the observed genetic predisposition to prostate cancer. Other segregation studies have suggested the existence of other prostate cancer susceptibility loci on chromosomes 1q42.2-43 (named *PCAP*)[40] 1p36 (named *CAPB,* also linked to brain tumors)[41] and Xq27-28[42], but the gene or genes linked to these regions have not been identified. More recent genome- wide scans in larger cohorts of hereditary prostate cancer families have identified additional chromosomal loci linked to prostate cancer, and it is likely that the number of known susceptibility genes will increase.

Prostate Cancer Susceptibility Genes

Gene	Chromosome Location	Year Identified	Function
ELAC2/HPC2	17p11	2001	Unknown
RNase L/*HPC1*	1q24-25	2002	Apoptosis and susceptibility to infection
SR-A/*MSR1*	8p22-23	2002	Inflammation and susceptibility to infection
OGG1	3p26.2	2002	DNA repair of oxidative damage
CHEK2	22q12.1	2003	DNA damage signaling and cell cycle control
BRCA2	13q12.3	2003	DNA repair
PON1	7q21.3	2003	Antioxidant, free radical scavenger
MIC1	19p13	2004	Inflammation

GENETIC ALTERATIONS IN PROSTATE CANCER

Epigenetic changes

Epigenetic events affect gene expression without altering the actual sequence of DNA. Known mechanisms include DNA hypermethylation and hypomethylation, chromatin remodeling, histone modification, and RNA interference [43].

A variety of genes implicated in prostate cancer initiation and progression are affected by these processes, including hypermethylation of hormonal response genes (*AR, ESR1, ESR2, RARB,* and *RARRES*), genes controlling the cell cycle (*CCND2* and *CDKN2A*), tumor cell invasion or tumor architecture genes (*APC, CAV1, CD44, CDH1, CDH13, LAMA3, LAMB3,* and *LAMC2*), DNA repair genes (*GSTP1* and *MGMT*), signal transduction genes (*DAB2IP, DAPK1, EDNRB,* and *RASSF1*), and inflammatory response genes (*PTGS2*); hypomethylation of *CAGE, HPSE,* and *PLAU;* histone hypoacetylation of *CAR, CPA3, RARB,* and vitamin D receptor; and histone methylation of *GSTP1* and PSA [44].

Clinical studies have shown that quantitative methylation analysis of the *GSTP1* gene can improve sensitivity and specificity for the diagnosis of cancer, and efforts to develop global gene methylation profiles in serum, urine, and tissue as an adjunct to predicting risk of cancer, determining the need for repeated biopsy, and demonstrating tumor aggressiveness are underway [45] .

Furthermore, agents that can inhibit or reverse the effects of DNA Methyltransferases and histone deacetylases and restore normal gene expression are under study for both prevention and therapy.

Somatic Mutations associated with Tumor Initiation and Progression

A number of somatic mutations in tumor DNA are acquired during clonal expansion of nascent tumors as a result of mitotic errors, defects in DNA repair mechanisms, loss of apoptotic ability, and other mechanisms. A variety of genes are so affected in prostate cancer.

Androgen Receptor

Polymorphisms of the androgen receptor are linked epidemiologically to prostate cancer risk. Mutations in the androgen receptor gene are present in about 50% of cancers and may cause

downregulation or loss of receptor expression, production of hypoactive or hyperactive receptors, alteration of receptor- ligand specificity, and conversion of androgen receptor antagonists to agonists, which may confer a growth advantage and permit tumor progression [46].

Many of the growth factors and tyrosine kinases implicated in prostate cancer initiation and progression may exert their effects through crosstalk with androgen receptor[47,48]. Recent work in xenografts demonstrates amplification of androgen receptor mRNA in hormone-refractory tumors, making "androgen-resistant" cells exquisitely sensitive to minute amounts of androgen[49]. This mechanism may underlie androgen resistance in men with end-stage disease, in which tumor progression invariably occurs despite castrate serum levels, and it may also explain some cases of paradoxical PSA declines after antiandrogen withdrawal (antiandrogen withdrawal syndrome)

DNA REPAIR GENE POLYMORPHISMS IN PROSTATE CANCER

No studies have examined the relationship between DNA repair capacity phenotypes and prostate cancer susceptibility, and only a few published studies on DNA repair genotypes and prostate cancer exist. Xu *et al.* studied 18 different genetic variants of the DNA repair enzyme gene *hOGG1*, involved in base excision repair, and found the genotype frequency of two sequence variants (11657*A/G* and *Ser*326*Cys*) was significantly different between prostate cancer cases and controls. They also confirmed the association with the 11657*A/G* variant in a family-based association study.

Van Gils *et al.*[50] showed that the *XRCC1* codon *Arg*399*Arg* genotype was associated with elevated prostate cancer risk in those with low vitamin E or lycopene intake. In linking genotypic risk with environmental exposure, the results of this study were consistent with the theory that DNA repair genes are most relevant in situations of high mutagenic exposures. There are currently over 100 known DNA repair genes, and most are known to have genetic variation in humans [51].

Rybicki et al examined the *XRCC1* codon 399 and *XPD* codons 312 and 751 polymorphisms in relation to prostate cancer risk in a large sample of primarily. Only the XPD codon 312 *Asn* allele showed a modest association with increased prostate cancer risk, 60%, when two copies of the allele were present. Perhaps more revealing, however, was the potential interaction between the *XPD* codon 312 *Asn* allele and the *XRCC1* codon 399 *Gln* allele. When both alleles were present in their homozygous states, the risk for prostate cancer increased 4.8-fold.

Whereas genetic interactions are thought to be more likely between genes involved in the same biological pathways, it is not unprecedented to find an increased joint effect between genes acting in different pathways. In the case of XRCC1 and XPD, because DNA damage caused by a mixture of environmental exposures may require either the base excision repair or nucleotide excision repair pathways, a reduction in the efficiencies of only one of these pathways may not increase disease risk to the same extent as when both pathways are compromised.

Previous studies of the *XRCC1* codon 399 polymorphism are equivocal with some finding increased risk for the *Gln* allele [52,53] but others finding an increased risk for the *Arg* allele [54, 55] .

MATERIAL AND METHODS

Study population : 50 patients who either attended the Urology Out patient Department or those admitted in Urology ward at GGH, Chennai.

Nature of Study : Retrospective study

No. of Ca.Prostate cases : 25

No. of Controls : 25

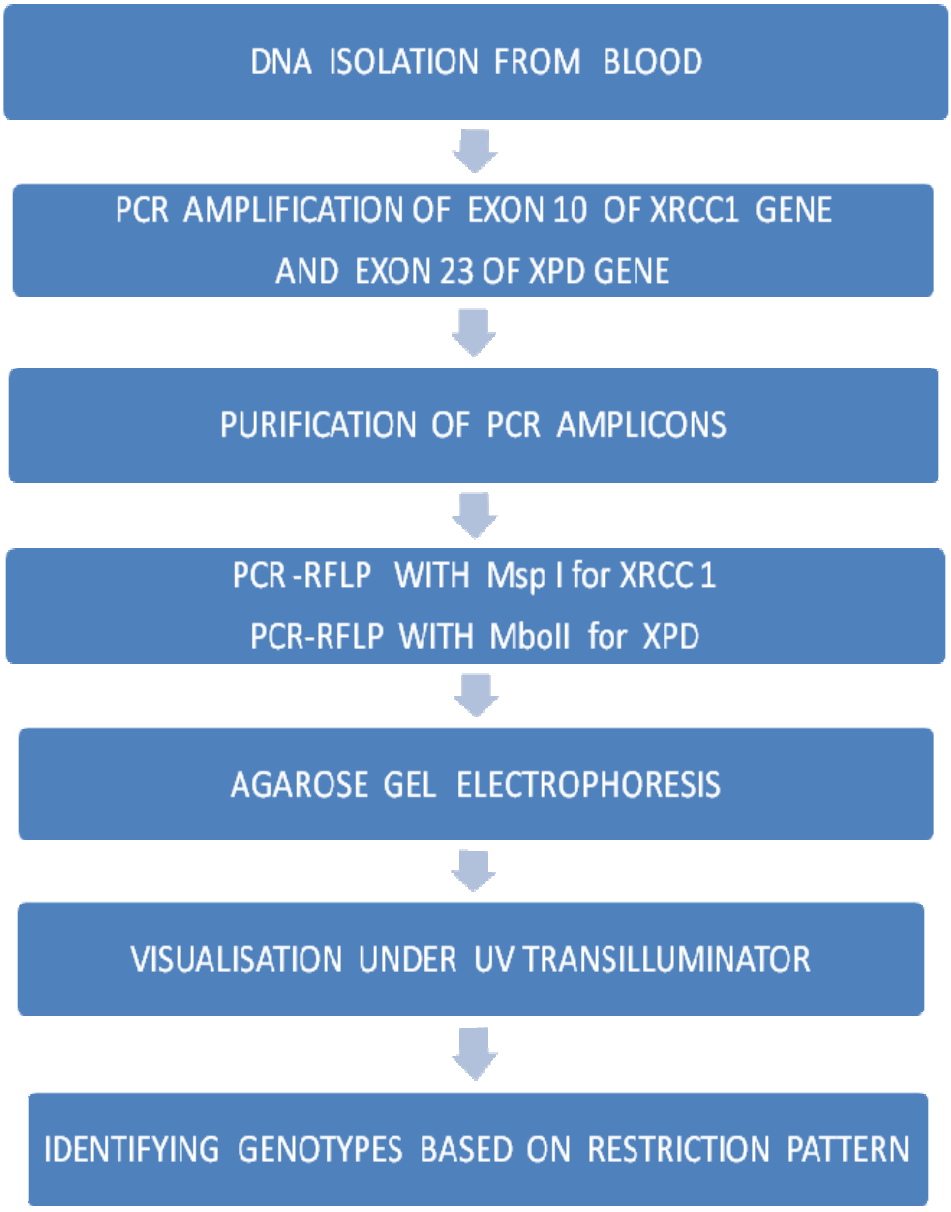
- BPH : 19
- Age matched controls: 6

Study Period : November 2008 - March 2011

The age of the subjects included in the study ranged from 55 to 87 years.

The cases and controls were similar in ethnicity and nutritional status. Clinical characteristics, including Gleason score, PSA, and tumor stage were obtained from the patients. The controls were healthy, unrelated individuals with normal serum PSA levels, normal digital rectal examination .

OUTLINE OF METHODOLOGY



BLOOD SAMPLE COLLECTION

5 ml of peripheral blood was obtained by direct venipuncture using disposable syringe from the antecubital vein of each subject. The samples were immediately transferred into EDTA containing vacutainer tubes. The vacutainer tubes were kept in thermos ice box and transferred immediately to the Department Endocrinology , IBMS, Taramani where the buffy coat was separated and stored at –20°C until isolation of DNA was done.

DNA ISOLATION AND PURIFICATION

The phenol chloroform method of DNA isolation was used in this study.

This frequently used method for DNA isolation removes proteins and other cellular components from nucleic acids, resulting in relatively pure DNA preparations.

I. PRINCIPLE

The concept of isolation of DNA is that all the other components of the cell and chromatin are removed using suitable methods to leave behind the DNA. In general the isolation of DNA from mammalian tissues follow four different steps.

1. Lysis of cells with a detergent like sodium dodecyl sulphate (SDS).

2. Digestion of proteins with enzymes (Proteinase – K).

3. Extraction of DNA by phenol chloroform method.

4. Precipitation of DNA with isopropyl alcohol or 100% ethanol.

II. REAGENTS AND THEIR FUNCTIONS

1. RBC Lysis Buffer

Ammonium chloride – 155mM (8.29g)

EDTA – 0.1mM (1.00g)

NaHCO3 – 12mM (0.034g)

pH adjusted to 7.4 with 1M HCl or NaOH, made upto 1000 ml with distilled water. Autoclaved and stored at room temperature.

The RBC lysis buffer was used to lyse the erythrocytes.

2. SE Buffer/WBC Lysis Buffer

Na2EDTA – 25mM (8.41g)

NaCl – 200mM (11.69g)

pH adjusted to 8.0 with 1M NaOH, made upto 1000 ml with distilled water, autoclaved and stored at room temperature.

3. Proteinase K (10 mg/ml)

Proteinase K - 100 mg

TE - 10 ml

100 mg Proteinase K dissolved in 10 ml TE for 30 min at room temperature and stored at -20°C. Proteinase K is the enzyme commonly employed for digestion of proteins. It is a highly active protease purified from the mold *Tritirachium album*. The digestion with proteinase K is usually carried out in presence of EDTA because EDTA inhibits the action of Mg^{2+} ion dependent nucleases which otherwise can digest the DNA.

4. Sodium dodecyl sulphate (SDS) 10%

SDS - 10 gram

Water added to make up to 100 ml, stirred on a magnetic stirrer, filtered and stored at room temperature.

SDS is the commonly used detergent for DNA isolation. It ruptures the cell wall and nuclear membranes to release the contents. Furthermore, it also denatures proteins present in the sample.

5. Phenol (Saturated, pH 8)

Phenol is used to extract the DNA from the solution. In alkaline pH it extracts the DNA to the aqueous phase, which is collected for further purification. This will prevent the contamination of DNA with RNAs. In neutralor acidic pH phenol extracts RNA to aqueous phase. Hence, the pH of phenol is very important for this step. The pH of phenol should be maintained above 7.8 as all eukayotic RNA with poly-A tails dissolve in alkaline phenol but in the acid range the DNA will partition into organic phase.

6. Phenol: Chloroform : Isoamyl alcohol mixture

Phenol: Chloroform: Isoamyl alcohol mixture prepared by mixing 25 parts of Phenol, 24 parts of Chloroform and 1 part of Isoamyl alcohol. The denaturation of proteins is mainly achieved through the activity of chloroform. It causes surface denaturation of proteins and also helps in removal of fats from the sample.

Chloroform also eliminates any traces of phenol as phenol can cause phosphodiester breakage. Mixture of phenol and chloroform is also useful for the removal of protein from nucleic acid samples. Because of the presence of proteins in the solution, the chance of foaming is more for the solution at the time of phenol: chloroform extraction. The action of isoamyl alcohol is to reduce the foaming and to maintain the stability of layers after centrifugation of deproteinised solution.

7. Isopropyl Alcohol

The action of isopropyl alcohol is to precipitate the DNA leaving RNA and polysaccharides in the solution.

8. 70% Ethanol

Ethanol	-	70 ml
Distilled water	-	30 ml1

It removes residual salt and moisture in the precipitated DNA.

9. Tris-EDTA (TE) Buffer (pH - 8.0)

Tris base	-	1.2114 gram
EDTA	-	0.0372 gram

Dissolved in 900 ml distilled water and the pH adjusted to 8. The volume made up to 1000 ml. Filtered , autoclaved and stored at 4^{o}C. Ideal buffer to store the DNA.

III. PROCEDURE

5ml of whole blood was taken and spun at 3500rpm for 20min at 25°C.

Buffy coat was removed carefully and transfered to a new 2.0 ml eppendorf tube. 1ml of 1x RBC lysis buffer was added to the buffy coat, vortexed, mixed well and incubated for 15 min at 37°C followed by a spin at 3500rpm for 15 min at room temperature. The supernatant was discarded, the pellet was dislodged and washed 2 or 3 times with 1ml RBC lysis buffer (repeat of step 2) until a half white pellet appears. The pellet was then dislodged by tapping to which 500µl of SE (WBC lysis) buffer, 5µl ProteinaseK (final concentration 200µg/ml) and 25µl (final concentration 0.5%) 10% SDS was added and incubated in water bath at 37°C for overnight or 55°C for 3 hours. Equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) to the lysate was added and intensely mixed well by inverting the tube until it turns to milky white in colour. The samples were spun at 10,000 rpm for 15min at room temperature. The upper aqueous phase alone was carefully collected with the help of wide

bore tips without disturbing the other layers and transferred to a new tube. To this aqueous phase 2.5 volumes of chilled absolute ethanol was added and the tubes were inverted gently for several times. The DNA will be visible like a thread and will assume the shape of a cotton ball. The DNA was transferred to an eppendorf tube already containing 1ml of 70% ethanol and spun at 12,000g for 10 min at 4°C. The Ethanol was discarded and the pellet is air-dried in a sterile place for 3 hours to remove any trace of residual ethanol.

Appropriate amount of 1X TE was added according to the size of the pellet, allowed to dissolve and stored at 4°C.

QUALITY CHECK & QUANTIFICATION OF DNA

The integrity of the DNA was assessed by running it in 0.7% Agarose gel. Further the quantification and quality check of DNA was performed by subjecting the DNA to spectrophotometry.

I. PRINCIPLE

The concept of quality check of DNA is to find out the purity of the extracted DNA. The extracted DNA may contain impurities like phenol, proteins and others. The integrity of the DNA is checked by agarose gel electrophoresis.

The DNA is mixed with loading dye and run electrophoretically on 0.7% agarose gel in TAE buffer, the high molecular weight DNA appeared as sharp band without smearing.

II. REAGENTS

1) TAE buffer (10x)

Tris base	-	48.4 gram
Glacial acetic acid	-	11.42 ml
0.5 M EDTA (pH 8.0)	-	20 ml

Distilled water added to make up to 1000 ml

Autoclaved and stored at room temperature.

2. Gel loading dye – Type III (6x)

Bromophenol blue	-	0.25% (w/v)
Xylene cyanol FF	-	0.25% (w/v)
Glycerol in water	-	30% (v/v)

Stirred well and stored at 4^{o}C.

3. Ethidium bromide

Ethidium bromide	-	10 mg
Distilled water	-	1 ml

Mixed well to ensure that the dye has dissolved completely. The tube wrapped in aluminum foil and stored at room temperature.

III. PROCEDURE FOR AGAROSE GEL ELECTROPHORESIS

1. 0.7% Agarose Gel preparation

- 0.7 gram of agarose weighed and transferred into a 250 ml conical flask.
- 100 ml of 0.5x TAE buffer was added to it, stirred well and melted on a magnetic stirrer cum hot plate until the agarose dissolves completely.
- The appropriate sized gel tray and comb was washed and wiped with 70% Ethanol. The gel tray was placed inside the casting unit. The comb was placed on the gel tray and left on an even surface.
- After the agarose cools down to hand bearing temperature, 5 µl of ethidium

  bromide was added and mixed well. It was poured on the gel tray and allowed to polymerize. After polymerization the comb is removed gently.

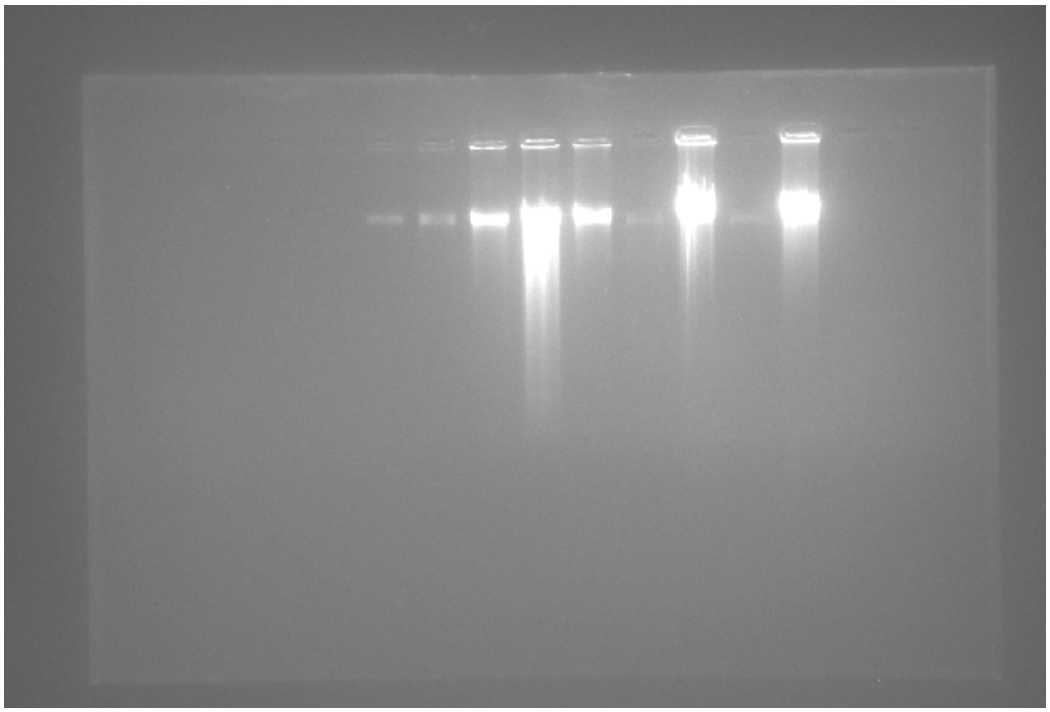
2. Preparation of sample and loading

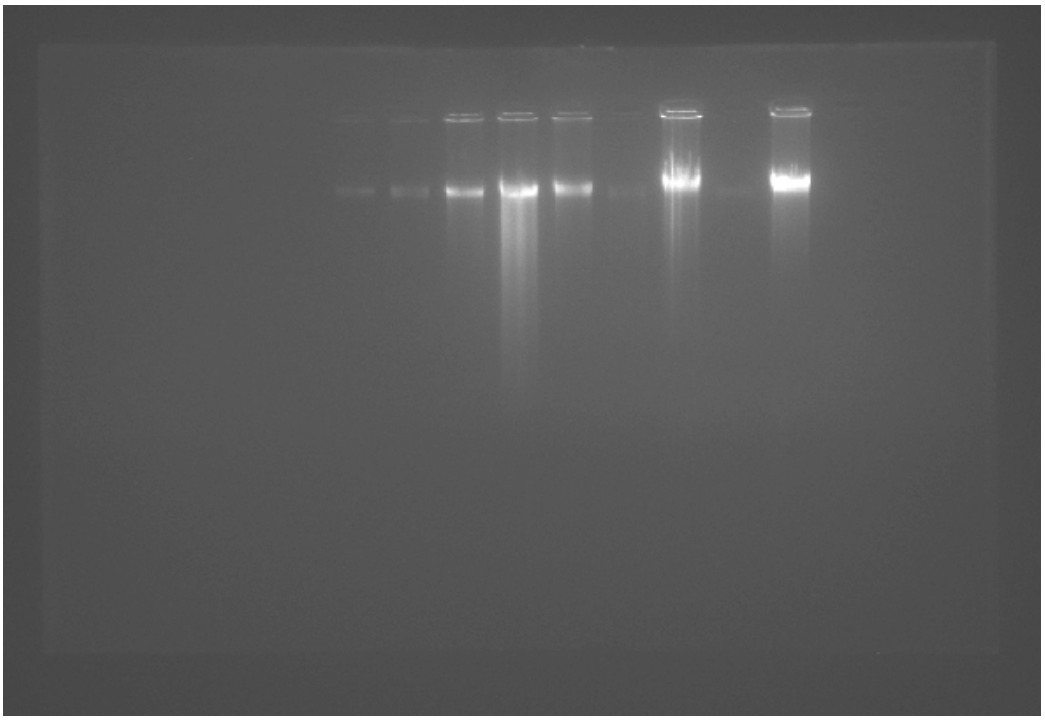
- The gel tray was removed from the casting unit and the tray placed in the electrophoresis tank.
- 0.5x TAE buffer was poured into the tank until the gel gets immersed.
- 2 µl of each DNA sample was taken and mixed with 2µl of 6x loading dye and 8ml of sterile double distilled water.
- The DNA samples were loaded into the wells.
- The electrodes were connected.
- The power was switched ON, set at 100 V.
- As the DNA is negatively charged, it will migrate towards the anode.

3. Visualizing the DNA

- When the bromophenol blue dye was in the middle of the gel, the power was switched OFF.
- The gel was taken to the transilluminator and observed under UV and documented.
- The good high molecular weight DNA will appear as sharp band without smearing.

DNA VISUALISED ON GEL





IV. PROCEDURE FOR SPECTROPHOTOMETRY

The nucleic acid sample was analysed at 260nm and 280nm by using Nanodrop Spectrophotometer (Thermo scientific, Germany). The concentration and purity of the sample was analysed using the following formula,

CONCENTRATION OF DNA:

Concentration of double stranded DNA sample (μg/μl) = A260 x 50

PURITY OF DNA:

Pure DNA = A260 /A280 ≥ 1.8

< 1.8 indicates protein and phenol contamination

>2.0 indicates the possible contamination with RNA

PRIMERS:

The PRIMERS were commercially procured. The forward and Reverse Sequences of the Primers for XPD and XRCC1 Exons are given below

PRIMERS FROM XPD EXON 23

FORWARD	5’-CAGGTGAGGGGGACATCTGG-3’
REVERSE	5’-CTCTCCCTTTCCTCTGTTCTCTGC-3’

PRIMERS FROM XRCC1 EXON 10

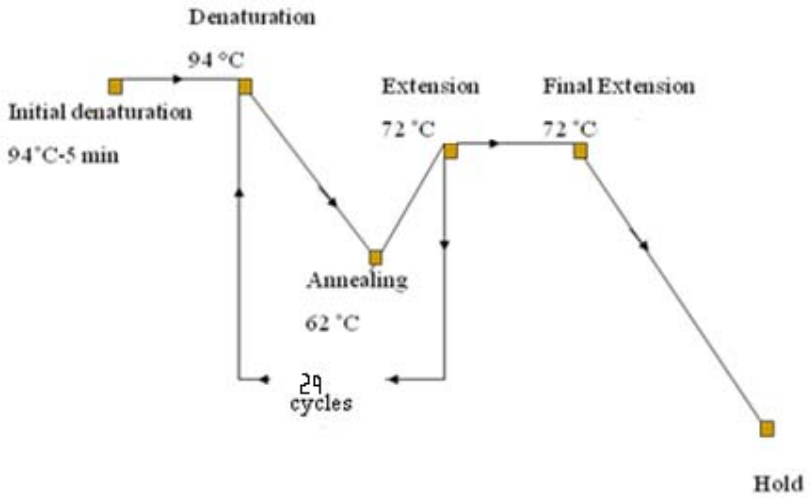
FORWARD	5'- TGCTTTCTCTGTGTCCACTATGCTGC-3'
REVERSE	5'- TCTGATAAGCAGGCTTCACAGAGCC-3'

Components of 10 µl PCR reaction mix

Contents	Volume	Final concentration
10 X PCR buffer II (Applied Biosystems (ABI), USA)	1 µl	1X
25mM $MgCl_2$ (ABI, USA)	1 µl	2.5 mM
2.5mM dNTP mix (TAKARA, Japan)	0.8 µl	0.2 mM
2 µM Forward Primer	0.5 µl	100 nM
2 µM Reverse Primer	0.5 µl	100 nM
(ABI, USA)		
5U/µl AmpliTaq ®DNAPol	0.05 µl	0.25 U/reaction
Template DNA(100ng/µl)	1 µl	100 ng/reaction
Sterile distilled water	5.15 µl	-
Total reaction volume	10 µl	-

POLYMERASE CHAIN REACTION

PCR thermal cycle (XRCC 1 EXON 10 AND XPD EXON 23)



STEPS	TEMPERATURE	TIME
Initial Denaturation	94 deg celcius	5 min
Denaturation	94	45 sec
Annealing	63	1 min cycle (29 cycles)
Extension	72	1 min
Final extension	72	5 min

Hold at 4 deg celcius.

After the completion of the thermal cycles, the PCR product was resolved on 2% Agarose gel in Mupid-ex electrophoresis tank (TAKARA, Japan), and the amplification was visualized and documented using UVP- UV Trans-illuminator.

PCR ANALYSIS

PCR- RFLP:

The PCR Fragment was digested by the enzyme MboII , which digests the 733 bp fragment of XPD Exon 10

Ends	Coordinates	Length (bp)
MboII-MboII	132-636	505
(LeftEnd)-MboII	1-131	131
MboII-(RightEnd)	637-733	97

Enzyme Msp I digests the PCR fragment of XRCC 1

#	Ends	Coordinates	Length (bp)
1	(LeftEnd)-MspI	1-372	372
2	MspI-(RightEnd)	373-599	227

Components for 20 µl RFLP Reaction mix

Contents	Volume	Final concentration
PCR product	10 µl	-
10X NE Buffer 4 (NEB Inc–USA)	2 µl	1 X
10X BSA (NEB Inc- USA)	2 µl	1 X
MspA1I (10 U/µl) (NEB Inc-USA)	0.2 µl	2 U/reaction
Sterile distilled water	5.8 µl	-
Total reaction volume	20 µl	-

RFLP reaction conditions

1. 37°C- 16 hours/Overnight

2. 4°C- ∞

After the completion of the restriction digestion, 20 µl of the digest product and 2 µl of 6X DNA gel loading dye was mixed well and resolved by electrophoresis in Mupid-ex electrophoresis tank (TAKARA, Japan) in 2.0% agarose gel. Theresolved gel was visualized using UVP-UV Trans-illuminator.

OBSERVATION AND RESULTS

STATISTICAL ANALYSIS

Statistical analysis was done using the SPSS Software.

The association between XRCC1 and XPD Polymorphisms and risk of Ca.Prostate was determined by calculating the Odds Ratio (OR) at 95% Confidence interval(CI).

Any possible effect modification by age was also evaluated by stratifying by age at diagnosis (<65 *versus* >65). In addition, to investigate the potential effect of genotype on disease aggressiveness, stratified the analyses of the cases' clinical characteristics at diagnosis done.The cancer risk was also analysed by stratifying the patients based on Gleason score and Sr.PSA levels.

TABLE - 1

ASSOCIATION OF XRCC 1
GENOTYPE AND PROSTATE CANCER

GENOTYPE	CASES (%) n- 25	CONTROLS(%) n - 25	OR (95% CI)	P
Arg/Arg	4 (16%)	10 (40%)	0.29 (0.06 – 1.27)	0.115
Arg/Gln	15(60%)	7 (28%)	3.86 (1.02 – 15.17)	0.046
Gln/Gln	6 (24%)	8 (32%)	0.67 (0.16 - 2.74)	0.753

The Association of XRCC 1 genotype and Prostate cancer was assessed in 25 Prostate cancer patients. 4 (16%) were Arg/Arg, 15 (60%) were Arg/Gln and 6 (24%) were Gln/Gln. Among the 25 controls, 10 (40%) were Arg/Arg, 7(28%) were Arg/Gln, 8 (32%) were Gln/Gln.

With Arg/Arg as the reference genotype , the Odds Ratio (95% CI) of the Homozygosity and heterozygosity of the Gln allele was assessed. It was found that the Arg/ Gln genotype was found to have statistically significant association with Prostate cancer ($p<0.05$).

The Arg/Arg genotype and Gln/Gln genotype did not have a significant association with Prostate cancer.

TABLE - 2

XRCC 1 GENOTYPE AMONG PROSTATE CANCER PATIENTS AND CONTROLS STRATIFIED BY AGE

GENOTYPE	CASES (%)	CONTROLS(%)	OR (95 % CI)	P
AGE < 65 YRS	N- 10	N- 14		
Arg/Arg	2(20%)	5(35.7%)	0.45 (0.04 – 3.99)	0.652
Arg/Gln	6(60%)	4(28.6%)	3.75 (0.51 – 31.14)	0.211
Gln/Gln	2(20%)	5(35.7%)	0.45 (0.04 – 3.99)	0.704
AGE ≥ 65 YRS	N- 15	N- 11		
Arg/Arg	2(13.3%)	5(45.4%)	0.18 (0.02 – 1.62)	0.169
Arg/Gln	9(60%)	3(27.2%)	4.00 (0.58 – 31.07)	0.209
Gln/Gln	4(26.6%)	3(27.2%)	0.97 (0.12 – 7.74)	1.000

The association of XRCC 1 genotype with Prostate cancer patients based on the age was calculated. Among the subjects less than 65 yrs, Arg/Arg was observed in 20% and 35.7%, Arg/Gln observed in 60% and 28.6%, Gln/Gln observed in 20 % and 35.7% of cases and controls respectively. Among the subjects more than 65 yrs, Arg/Arg was observed in 13.3% and 45.4%, Arg/Gln in 60% and 27.2% , Gln/Gln in 26.6% and 27.2% of cases and controls respectively. No significant association was observed between the genotypes stratified by age and risk of Prostate cancer.

XRCC 1 GENOTYPE AMONG PROSTATE CANCER PATIENTS AND CONTROLS STRATIFIED BY AGE

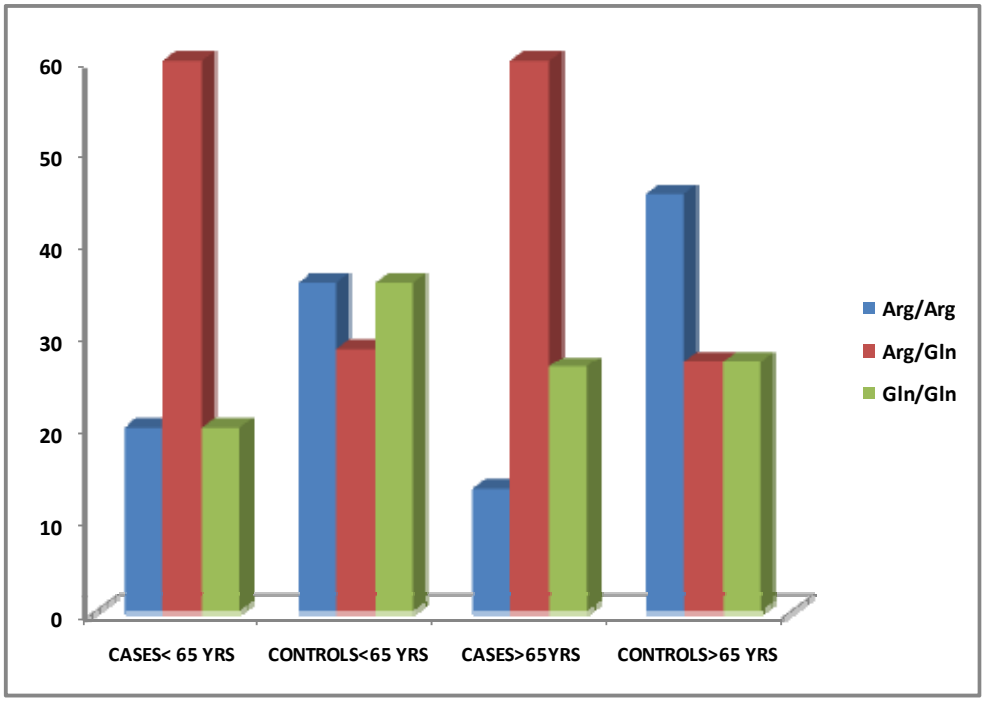


TABLE - 3

ASSOCIATION OF XRCC 1 GENE WITH GLEASON SCORE IN PATIENTS

GENOTYPE	PATIENTS(%)		OR	95% CI	P
	GLEASON SCORE≥7 n- 12	GLEASON SCORE<7 n-13			
Arg/Arg	2(16.6)	2(15.3)	1.10	0.09 – 14.18	1.000
Arg/Gln	8(66.6)	7(53.8)	1.71	0.26 – 11.92	0.688
Gln/Gln	2(16.6)	4(30.7)	0.45	0.04 – 4.10	0.645

The XRCC 1 genotype distribution based on the Gleason score was assessed.

Arg/Arg genotype was seen in 16.6% and 15.3% , Arg/Gln genotype in 66.6% and 53.8%, Gln/Gln in 16.6% and 30.7% of

patients with Gleason score less than 7 and more than 7 respectively. No significant association between the genotypes and Histologic grade of Prostate cancer. Statistical assessment is limited by the small number of patients in each group.

TABLE - 4

DISTRIBUTION OF XRCC 1 GENOTYPE IN PATIENTS BASED ON PSA LEVELS

GENOTYPE	PATIENTS(%)		OR	95% CI	P
	PSA ≥50 ng/dl n-10	PSA<50 ng/dl n-15			
Arg/Arg	2(20)	2(13.3)	1.00	0.13 – 21.44	1.000
Arg/Gln	6(60)	9(60)	1.00	0.15 – 6.91	1.000
Gln/Gln	2(20)	4(26.6)	0.69	0.07 – 6.38	1.000

The XRCC 1 Genotype association with Prostate cancer based on the Sr.PSA levels were assessed. Arg/ Arg genotype was observed in 20% and 13.3 % , Arg/Gln in 60% and 60% , Gln/Gln in 20% and 26.6% of patients with Sr. PSA levels of more than 50 ng/ml and

less than 50 ng/ml respectively. No significant association was observed between the genotypes stratified by Serum PSA levels and Carcinoma Prostate.

TABLE 5

ASSOCIATION OF XPD GENOTYPE AND PROSTATE CANCER

GENOTYPE	CASES (%) n- 25	CONTROLS(%) n - 25	OR (95% CI)	P
Lys/Lys	9 (36%)	12 (48%)	0.61 (0.17 – 2.19)	0.567
Lys/Gln	13(52%)	5 (20%)	4.33 (1.06 – 18.63)	0.039
Gln/Gln	3(12%)	8(32%)	0.29 (0.05 – 1.49)	0.172

The Association of XPD genotype and Prostate cancer was assessed in 25 Prostate cancer patients . 9 (36%) were Lys/Lys , 13(52%) were Lys/Gln and 3 (12%) were Gln/Gln. Among the 25 controls, 12 (48%) were Lys/Lys , 5(20%) were Lys/Gln , 8 (32%) were Gln/Gln.With Lys/Lys as the reference genotype , the Odds Ratio (95% CI) of the Homozygosity and heterozygosity of the Gln allele was assessed .It was found that the Lys / Gln genotype was found to have statistically significant association with Prostate cancer

($p<0.05$). The Lys/Lys genotype and Gln/Gln genotype did not have a significant association with Prostate cancer.

TABLE - 6

XPD GENOTYPE AMONG PROSTATE CANCER PATIENTS AND CONTROLS STRATIFIED BY AGE

GENOTYPE	CASES (%)	CONTROLS(%)	OR	95 % CI
AGE < 65 YRS	N- 10	N- 14		
Lys/Lys	4(40%)	6(42.8%)	0.89 (0.13 – 6.25)	1.000
Lys/Gln	5(50%)	3(21.4%)	3.67 (0.46 –32.64)	0.204
Gln/Gln	1(10%)	5(35.7%)	0.20 (0.01 – 2.55)	0.339
AGE ≥ 65 YRS	N- 15	N- 11		
Lys/Lys	5(33.3%)	6(54.5%)	0.42 (0.06 – 2.69)	0.426
Lys/Gln	8(53.3%)	2(18.1%)	5.14 (0.64 –50.53)	0.157
Gln/Gln	2(13.3%)	3(27.2%)	0.41 (0.04 – 4.13)	0.698

The association of XPD genotype with Prostate cancer patients based on the age was calculated. Among the subjects less than 65 yrs, Lys/Lys was observed in 40% and 42.8 % , Lys/Gln observed in 50% and 21.4% , Gln/Gln observed in 10 % and 35.7% of cases

and controls respectively. Among the subjects more than 65 yrs , Lys/Lys was observed in 33.3% and 54.5% , Lys/Gln in 53.3% and 18.1% , Gln/Gln in 13.3% and 27.2% of cases and controls respectively. No significant association was observed between the genotypes stratified by age and risk of Prostate cancer.

XPD GENOTYPE AMONG PROSTATE CANCER PATIENTS AND CONTROLS STRATIFIED BY AGE

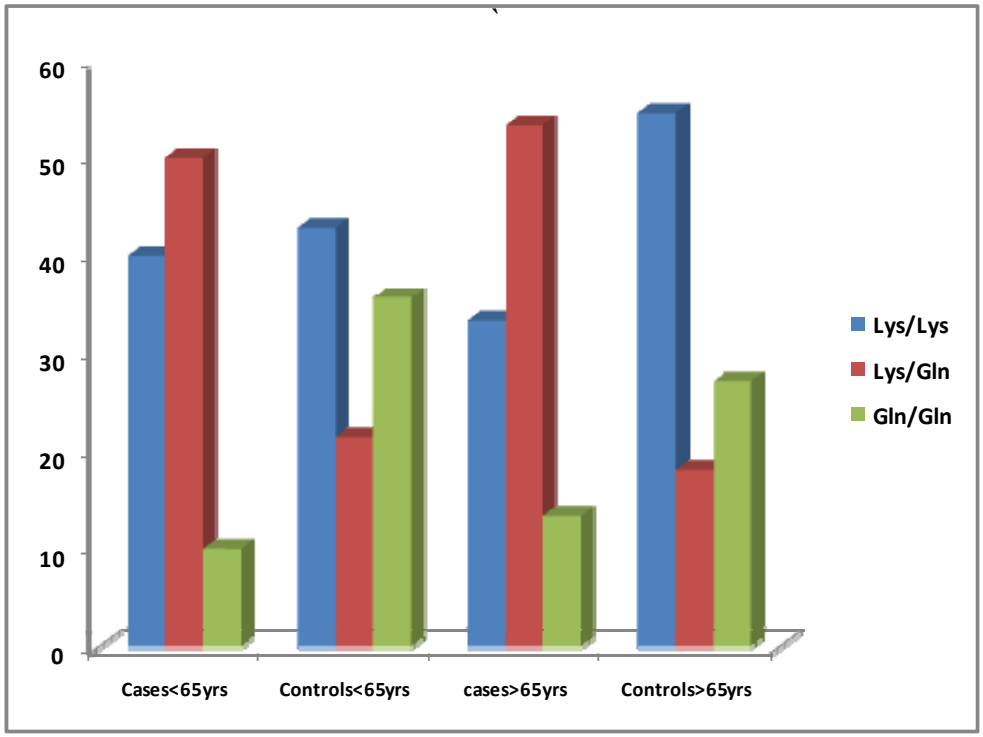


TABLE - 7

ASSOCIATION OF XPD GENE AND GLEASON SCORE

GENOTYPE	PATIENTS(%)		OR	95% CI	P
	GLEASON SCORE≥7 n- 12	GLEASON SCORE<7 n-13			
Lys/Lys	5(41.6)	4(30.7)	1.61	0.23 – 11.45	0.688
Lys/Gln	6(50)	7(53.8)	0.86	0.13 – 5.47	0.835
Gln/Gln	1(8.3)	2(15.3)	0.50	0.02 – 8.95	1.000

The XPD genotype distribution based on the Gleason score was assessed.

Lys/Lys genotype was seen in 41.6% and 30.7% , Ly/Gln genotype in 50% and 53.8% , Gln/Gln in 8.3% and 15.3% of patients with Gleason score less than 7 and more than 7 respectively. There was no significant association between the genotypes and Histologic grade of Prostate cancer. Statistical

assessment is limited by the small number of patients in each group.

TABLE - 8

DISTRIBUTION OF XPD GENOTYPE IN PATIENTS BASED ON PSA LEVELS

GENOTYPE	PATIENTS(%)		OR	95% CI	P
	PSA ≥50 ng/dl n-10	PSA<50 ng/dl n-15			
Lys/Lys	4(40)	5(33.3)	1.33	0.19 – 9.59	1.000
Lys/Gln	5(50)	8(53.3)	0.88	0.13 – 5.79	1.000
Gln/Gln	1(10)	2(13.3)	0.72	0.02 – 13.11	1.000

The XPD Genotype association with Prostate cancer based on theSr.PSA levels were assessed. Lys/Lys genotype was observed in 40% and 33.3 % , Lys/Gln in 50% and 53.3% , Gln/Gln in 10% and 13.3% of patients with Sr. PSA levels of more than 50 ng/ml and less than 50 ng/ml respectively.

No significant association was observed between the genotypes stratified by Serum PSA levels and Carcinoma Prostate.

DISCUSSION

The Arg/ Gln genotype in XRCC1 and Lys/Gln genotype in XPD were found in 60 % and 52 % of Ca.Prostate patients and found to be statistically significant with the risk of Prostate cancer. All the subjects included in our study were unrelated. The other genotypes did not show a significant risk of developing Ca.Prostate.

The genotype association with Ca.Prostate based on the age, Gleason score and Sr.PSA was not found to be statistically significant. The statistical assessment is limited by the small number of patients .

Rybicki et al examined the *XRCC1* codon 399 and *XPD* codons 312 and 751 polymorphisms in relation to prostate cancer risk in a large sample of primarily Caucasian sibships. Only the XPD codon 312 *Asn* allele showed a modest association with increased prostate cancer risk, 60%, when two copies of the allele were present. Perhaps more revealing, however, was the potential interaction between the *XPD* codon 312 *Asn* allele and the *XRCC1* codon 399 *Gln* allele. When both

alleles were present in their homozygous states, the risk for prostate cancer increased 4.8-fold.

Only a few studies of more common DNA repair genetic variants and prostate cancer risk exist in the literature. Xu *et al.* studied 18 different genetic variants of the DNA repair enzyme gene *hOGG1*, involved in base excision repair, and found the genotype frequency of two sequence variants (11657*A/G* and *Ser*326*Cys*) was significantly different between prostate cancer cases and controls. They also confirmed the association with the 11657*A/G* variant in a family-based association study. Van Gils *et al* [50] studied three genetic variants in another base excision repair enzyme gene, *XRCC1,* and found no association between *XRCC1* polymorphisms and prostate cancer when only comparing genotype frequencies in cases and controls. However, when they stratified the study population by intake of several different dietary antioxidants, the more common *XRCC1* codon 399 *Arg/Arg* genotype was associated with prostate cancer in those with low vitamin E or lycopene intake.

Rybicki et al , on the other hand, found the less common *XRCC1* codon 399 *Gln*/*Gln* genotype to be a potential modifying factor for prostate cancer risk associated with the *XPD* codon 312 *Asn*/ *Asn*

genotype. It is not inconceivable that interactions at the *XRCC1* codon 399 locus are dependent on genotype, with some genetic or environmental risk factors preferentially interacting with the *Arg/Arg* genotype and others more likely to interact with the *Gln/Gln* genotype. The unadjusted ORs for the *XRCC1* codon 399 *Gln/Gln* genotype in the study of van Gils *et al.* was 0.77, compared with Rybicki et al OR estimate of 0.88. Previous studies of the *XRCC1* codon 399 polymorphism are equivocal with some finding increased risk for the *Gln* allele but others finding an increased risk for the *Arg* allele . Rybicki et al family-based study had several strengths, which include the size of the study population, the elimination of potential bias due to population genetic substructure, and full utilization of sibship data (without parental genotypes) that were composed of numerous configurations including sibships with only affected brothers.

The incorporation of genotypic information of unaffected brothers for a common disease such as prostate cancer can also significantly increase statistical power [56]. Another advantage of a family-based design concerns the absence of Hardy-Weinberg equilibrium in a control population found for the *XPD* codon 312 polymorphism. Although this would be troublesome in a case-control population, it was less of a concern in Rybicki et al family-based study because it could

be due to the association of this genotype with prostate cancer-affected brothers of these controls.

Despite the many advantages of family-based population, several potential disadvantages also exist including the potential for selection bias due to difficulty of enrolling multiple family members [57] and decreased statistical power compared with case-control populations under some circumstances [58, 59.]

Rybicki et al found, that when stratifying on family history, the risk associated with the *XPD* codon 312 *Asn/Asn* genotype was greater in siblings with a negative family history, which would suggest that the population risk for this genotype may be greater than reported.

CONCLUSION

The XRCC 1 Arg/Gln genotype and XPD Lys/Gln genotype were significantly associated with an increased risk of developing Prostate cancer.

The XRCC 1 and XPD genotypes stratified by age, grade and Sr.PSA levels did not show any significant risk of developing Prostate cancer.

The present study of DNA Repair gene polymorphisms predicts risk of developing Prostate cancer and would enable identification of genetically predisposed individuals.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. I.C. Iyal Amuthan
PG in MCh Urology
Madras Medical College, Chennai -3

Dear Dr. I.C. Iyal Amuthan

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the project / proposal / clinical trail entitled "The Association of DNA repair Gene Polymorhisms with Carcinoma Prostate" No. 06102010.

The following members of Ethics Committee were present in the meeting held on 22.10.2010 conducted at Madras Medical College, Chennai -3.

1. Prof. S.K. Rajan, MD	-- Chairperson
2. Prof. J. Mohanasundaram, MD,Ph.D,DNB Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3. Prof. A. Sundaram, MD Vice Principal , MMC, Chennai -3	-- Member Secretary
4. Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
5. Prof. Pregna B. Dolia , MD Director, Institute of Biochemistry, MMC, Ch-3	-- Member
6. Prof. C. Rajendran , MD Director, Institute of Internal Medicine, MMC, Ch-3	-- Member
7. Prof. Md. Ali, MD, DM Professor & Head ,,Dept. of MGE, MMC, Ch-3	-- Member
8. Thiru. S. Govindasamy BA.BL	-- Lawyer
9. Tmt. Arnold Soulina	-- Social Scientist

We approve the Proposal to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

MASTER CHART

Patient	Age (Yr)	S.PSA (ng/ml)	Diagnosis	HPE	Gleason Score
1	65	13.99	CA PROSTATE	ADENOCARCINOMA	4
2	67	5.16	BPH	BPH	
3	56	7.36	CA PROSTATE	ADENOCARCINOMA	5
4	63	9.98	CA PROSTATE	ADENOCARCINOMA	4
5	77	9.77	CA PROSTATE	ADENOCARCINOMA	3
6	70	3.26	BPH	BPH	
7	69	4.14	BPH	BPH	
8	64	22.14	CA PROSTATE	ADENOCARCINOMA	5
9	80	89.36	CA PROSTATE	ADENOCARCINOMA	8
10	55	6.96	BPH	BPH	
11	60	7.14	BPH	BPH	
12	64		CONTROL		
13	63	2.76	BPH	BPH	
14	83	122.41	CA PROSTATE	ADENOCARCINOMA	9
15	61	78.16	CA PROSTATE	ADENOCARCINOMA	7
16	60	156.42	CA PROSTATE	ADENOCARCINOMA	8
17	62	4.14	BPH	BPH	
18	61	3.32	BPH	BPH	
19	59	6.69	BPH	BPH	
20	63	16.44	CA PROSTATE	ADENOCARCINOMA	7
21	63		CONTROL		
22	87	246.56	CA PROSTATE	ADENOCARCINOMA	9
23	65	7.24	BPH	BPH	
24	56	2.14	BPH	BPH	
25	70	3.67	BPH	BPH	

Patient	Age (Yr)	S.PSA (ng/ml)	Diagnosis	HPE	Gleason Score
26	84	272.47	CA PROSTATE	ADENOCARCINOMA	8
27	72	5.17	BPH	BPH	
28	62		CONTROL		
29	81	37.27	CA PROSTATE	ADENOCARCINOMA	7
30	67	4.24	BPH	BPH	
31	61		CONTROL		
32	70	48.56	CA PROSTATE	ADENOCARCINOMA	6
33	61	19.96	CA PROSTATE	ADENOCARCINOMA	4
34	69	7.42	BPH	BPH	
35	73	98.17	CA PROSTATE	ADENOCARCINOMA	8
36	77	72.74	CA PROSTATE	ADENOCARCINOMA	8
37	73	8.19	BPH	BPH	
38	74	46.14	CA PROSTATE	ADENOCARCINOMA	6
39	62	3.27	BPH	BPH	
40	69	89.86	CA PROSTATE	ADENOCARCINOMA	8
41	78	15.45	CA PROSTATE	ADENOCARCINOMA	5
42	64		CONTROL		
43	62	29.32	CA PROSTATE	ADENOCARCINOMA	6
44	72		CONTROL		
45	64	17.14	CA PROSTATE	ADENOCARCINOMA	4
46	74	4.12	BPH	BPH	
47	71	104.42	CA PROSTATE	ADENOCARCINOMA	8
48	68	3.89	BPH	BPH	
49	63	23.32	CA PROSTATE	ADENOCARCINOMA	4
50	76	36.16	CA PROSTATE	ADENOCARCINOMA	5

PROFORMA

NAME:

AGE :

ADDRESS:

CONTACT NUMBER:

IP NO./ OP NO.:

EXAMINATION:

-DIGITAL RECTAL EXAMINATION

INVESTIGATIONS:

- Sr.PSA
- TRUS GUIDED BIOPSY

STUDY TYPE : Retrospective study

Retrospective analysis of Carcinoma Prostate patients , BPH and age matched controls done

.

INCLUSION CRITERIA: Patients with TRUS biopsy proven Carcinoma prostate and Controls (includes BPH patients and age adjusted controls)

METHOD:

- Isolation of DNA from peripheral blood sample by Phenol chloroform method
- DNA Precipitation and purification
- PCR amplification of DNA repair genes.
- Purification of PCR Product.
- PCR-RFLP
- Analysis of the association of the Genotypes of XRCC 1 and XPD with the cases and controls.

STATISTICAL ANALYSIS :

Statistical analysis done using the SPSS Software. The Odds Ratio, Confidence interval and P value assessed for association and risk of Prostate cancer with the various genotypes of XRCC 1 and XPD Genes.

BIBLIOGRAPHY

1. Carter, B. S., Beaty, T. H., Steinberg, G. D., Childs, B., and Walsh, P. C. Mendelian inheritance of familial prostate cancer. Proc. Natl. Acad. Sci. USA,1992; *89:* 3367–3371

2. Coughlin, S. S., and Hall, I. J. A review of genetic polymorphisms and prostate cancer risk. Ann. Epidemiol. 2002; *12:* 182–196.

3. Nwosu, V., Carpten, J., Trent, J. M., and Sheridan, R. Heterogeneity of genetic alterations in prostate cancer: evidence of the complex nature of the disease. Hum. Mol. Genet.2001; *10:* 2313–2318.

4. Bova, G. S., and Isaacs, W. B. Review of allelic loss and gain in prostate cancer. World J. Urol.1996; *14:* 338–346.

5. Dong, J. T., Suzuki, H., Pin, S. S., Bova, G. S., Schalken, J. A., Isaacs, W. B., Barrett, J. C., and Isaacs, J. T. Down-regulation of the KAI1 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Res.1996; *56:* 4387–4390.

6. Wang, C. Y., Debiec-Rychter, M., Schut, H. A., Morse, P., Jones, R. F., Archer, C., King, C. M., and Haas, G. P. N-Acetyltransferase expression and DNA binding of N-hydroxyheterocyclic amines in human prostate epithelium. Carcinogenesis (Lond.).1999; *20:* 1591–1595.

7. Nelson, C. P., Kidd, L. C., Sauvageot, J., Isaacs, W. B., De Marzo, A. M., Groopman, J. D., Nelson, W. G., and Kensler, T. W. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione *S*-transferase P1. Cancer Res.2001;*61:* 103–109.

8. Fleshner, N. E., and Kucuk, O. Antioxidant dietary supplements: Rationale and current status as chemopreventive agents for prostate cancer. Urology 2001; *57:* 90–94.

9. Caldecott, K. W., Tucker, J. D., Stanker, L. H., and Thompson, L. H. Characterization of the XRCC1-DNA ligase III complex *in vitro* and its absence from mutant hamster cells. Nucleic Acids Res.1995; *23:* 4836–4843.

10. Dianov, G. L., Prasad, R., Wilson, S. H., and Bohr, V. A. Role of DNA polymerase _ in the excision step of long patch mammalian base excision repair. J. Biol. Chem.1999; *274:* 13741–13743.

11. Thompson, L. H., and West, M. G. XRCC1 keeps DNA from getting stranded. Mutat. Res.2000; *459:* 1–18.

12. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol. Cell. Biol.1998; *18:* 3563–3571.

13. Zhang, Y., Weksler, B., Wang, L., Schwartz, J., and Santella, R. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. Atherosclerosis.1998;*140:* 325–331.

14. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res.1999; *59:* 2557–2561.

15. Duell, E. J., Wiencke, J. K., Cheng, T., Varkonyi, A., Zuo, Z., Ashock, T. D., Mark, E. J., Wain, J. C., Christiani, D. C., and Kelsey, K. T. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis (Lond.).2000; *21:* 965–971.

16. Abdel-Rahman, S. Z., and El-Zein, R. A. The 399Gln polymorphism in the DNA repair gene *XRCC1* modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. Cancer Lett.2000; *159:* 63–71.

17. Evans, E., Moggs, J. G., Hwang, J. R., Egly, J. M., and Wood, R. D. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. EMBO J.1997;*16:* 6559–6573.

18. Moncollin, V., Vichi, P., and Egly, J. M. TFIIH: a transcription factor involved in DNA repair and cell-cycle regulation. *In:* J. A. Nickoloffn and M. F. Hoekstra (eds.), DNA Damage and Repair, pp. 143–159. Totowa, New Jersey:

19. Quinn M, Babb P: Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: International comparisons. *Br J Urol Intl* 2002; 90:162-173.

20. Gronberg H: Prostate cancer epidemiology. *Lancet* 2003; 361:859-864.

21. Muir CS, Nectoux J, Staszewski J: The epidemiology of prostatic cancer. Geographical distribution and time-trends. *Acta Oncol* 1991; 30:133-140.

22. Shimizu H, Ross RK, Bernstein L, et al: Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. *Br J Cancer* 1991; 63:963-966.

23. Landis SH, Murray T, Bolden S, Wingo PA: Cancer statistics, 1999. *CA Cancer J Clin* 1999; 49:8-31.

24. Ries et al., 2004. In: Ries LAG, Eisner MP, Kosary CL, et al ed. *SEER Cancer Statistics Review, 1975-2001*, Bethesda, Md: National Cancer Institute; 2004.

25. Sakr WA, Haas GP, Cassin BF, et al: The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. *J Urol* 1993; 150:379-385.

26. Hankey BF, Feuer EJ, Clegg LX, et al: Cancer surveillance series: Interpreting trends in prostate cancer—part I: Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates. *J Natl Cancer Inst* 1999; 91:1017-1024.

27. Woolf C: An investigation of the familial aspects of carcinoma of the prostate. *Cancer* 1960; 13:739-744.

28, Eeles RA, Dearnaley DP, Ardern-Jones A, et al: Familial prostate cancer: The evidence and the Cancer Research Campaign/British Prostate Group (CRC/BPG) UK Familial Prostate Cancer Study. *Br J Urol* 1997; 79(suppl 1):8-14.

29. Ahlbom A, Lichtenstein P, Malmstom H, et al: Cancer in twins: Genetic and nongenetic familial risk factors. *J Natl Cancer Inst* 1994; 89:287-293.

30. Page W, Braun M, Partin A, et al: Heredity and prostate cancer: A study of World War II veteran twins. *Prostate* 1997; 33:240-245.

31. Gillanders EM, Xu J, Chang B, et al: Combined genome-wide scan for prostate cancer susceptibility genes. *J Natl Cancer Inst* 2004; 96:1240-1247.

32. Carpten J, Nupponen N, Isaacs S, et al: Germline mutations in the ribonuclease L (RNase L) gene in hereditary prostate cancer 1 (HPC1)–linked families. *Nat Genet* 2002; 30:181-184.

33. Tavtigian SV, Simard J, Teng DH, et al: A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet* 2001; 27:172-180.

34. Xu J, Zheng SL, Turner A, et al: Associations between hOGG1 sequence variants and prostate cancer susceptibility. *Cancer Res* 2002; 62:2253-2257.

35. Dong X, Wang L, Taniguchi K, et al: Mutations in CHEK2 associated with prostate cancer risk. *Am J Hum Genet* 2003; 72:270-280.

36. Edwards SM, Kote-Jarai Z, Meitz J, et al: Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. *Am J Hum Genet* 2003; 72:1-12.

37. Marchesani M, Hakkarainen A, Tuomainen TP, et al: New paraoxonase 1 polymorphism I102V and the risk of prostate cancer in Finnish men. *J Natl Cancer Inst* 2003; 95:812-818.

38. Xu J, Zheng SL, Turner A, et al: Associations between hOGG1 sequence variants and prostate cancer susceptibility. *Cancer Res* 2002; 62:2253-2257.

39. Lindmark F, Zheng SL, Wiklund F, et al: H6D polymorphism in macrophage-inhibitory cytokine-1 gene associated with prostate cancer. *J Natl Cancer Inst* 2004; 96:1248-1254.

40. Berthon P, Valeri A, Cohen-Akenine A, et al: Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43. *Am J Hum Genet* 1998; 62:1416-1420.

41. Gibbs M, Stanford J, McIndoe R, et al: Evidence for a rare prostate cancer susceptibility locus at chromosome 1p36. *Am J Hum Genet* 1999; 64:776-780.

42. Xu J, Meyers D, Freije D, et al: Evidence for a prostate cancer susceptibility locus on the X chromosome. *Nat Genet* 1998; 20:175-178.

43. Gonzalgo ML, Isaacs WB: Molecular pathways to prostate cancer. *J Urol* 2003; 170:2444-2452.

44. Long-Cheng L, Carroll PR, Dahiya R: Epigenetic changes in prostate cancer: Implication for diagnosis and treatment. *J Natl Cancer Inst* 2005; 97:103-115.

45. Harden SV, Sanderson H, Goodman SN, et al: Quantitative GSTP1 methylation and the detection of prostate adenocarcinoma in sextant biopsies. *J Natl Cancer Inst* 2003; 95:1634-1637.

46. Bostwick DG, Burke HB, Djakiew D, et al: Human prostate cancer risk factors. *Cancer* 2004; 101:2371-2490.

47. Culig Z, Hobisch A, Cronauer MV, et al: Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res* 1994; 54:5474-5479.

48. Craft N, Shostak Y, Carey M, Sawyers CL: A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat Med* 1999; 5:280-283.

49. Chen CD, Welsbie DS, Tran C, et al: Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004; 10:33-39.

50. Van Gils, C. H., Bostick, R. M., Stern, M. C., and Taylor, J. A. Differences in base excision repair capacity may modulate the effect of dietary antioxidant intake on prostate cancer risk: an example of polymorphisms in the *XRCC1* gene. Cancer Epidemiol. Biomark. Prev.2002; *11:* 1279–1284.

51. Wood, R. D., Mitchell, M., Sgouros, J., and Lindahl, T. Human DNA repair genes. Science (Wash. DC),2001; *291:* 1284–1289.

52. Sturgis, E. M., Castillo, E. J., Li, L., Zheng, R., Eicher, S. A., Clayman, G. L., Strom, S. S., Spitz, M. R., and Wei, Q. Polymorphisms of DNA repair gene *XRCC1* in squamous cell carcinoma of the head and neck. Carcinogenesis (Lond.), 1999;*20:* 2125–2129.

53. Duell, E. J., Millikan, R. C., Pittman, G. S., Winkel, S., Lunn, R. M., Tse, C. K., Eaton, A., Mohrenweiser, H. W., Newman, B., and Bell, D. A. Polymorphisms in the DNA repair gene *XRCC1* and breast cancer. Cancer Epidemiol. Biomark. Prev.2001; *10:* 217–222.

54. Olshan, A. F., Watson, M. A., Weissler, M. C., and Bell, D. A. XRCC1 polymorphisms and head and neck cancer. Cancer Lett.2002; *178:* 181–186.

55. Nelson, H. H., Kelsey, K. T., Mott, L. A., and Karagas, M. R. The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer: evidence of gene-environment interaction. Cancer Res.2002; *62:* 152–155.

56. Horvath, S., and Laird, N. M. A discordant-sibship test for disequilibrium and linkage: no need for parental data. Am. J. Hum. Genet.1998; *63:* 1886–1897.

57. Schulze, T. G., Muller, D. J., Krauss, H., Gross, M., Bauer, I., Fangerau-Lefevre, H., Illes, F., Ohlraun, S., Fimmers, R., Cichon, S., Held, T., Propping, P., Nothen, M. M., Maier, W., and Rietschel, M. Caught in the trio trap? Potential selection bias inherent to association studies using parent-offspring trios. Am. J. Med. Genet.2001; *105:* 351–353.

58. Risch, N., and Teng, J. The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases I. DNA pooling. Genome Res.1998; *8:* 1273–1288.

59. Schaid, D. J., and Rowland, C. Use of parents, sibs, and unrelated controls for detection of associations between genetic markers and disease. Am. J. Hum. Genet.1998; *63:* 1492–1506.