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The Relation between GSTP1 Gene Polymorphism and Prostate Cancer

Soujanya Ghosh*, Dr. Anindya Dasgupta

Biotechnologist and clinical researcher, Haldia Institute of Technology, India
HOD & Professor of Biochemistry Department, Calcutta National Medical College and Hospital, India

Abstract

Background: GSTP1 gene is present in the 11th chromosome. It functionally encodes different Glutathione S- transferase Pi 1 (GSTP1) variant proteins that are thought to function in xenobiotic metabolism and play a role in preventing prostate cancer. This gene suppress tumour genesis by detoxifying toxic carcinogens and reactive oxygen species (ROS). Prostate cancer is related to several mutations affecting the expression of GSTP1 (365 bp long). A single nucleotide polymorphism (SNP) in the GSTP1 gene results insignificant reduction in its anticancer activity due to the accumulation of toxic elements.

Basic framework: At first different blood samples are taken from different patients of prostate cancer. DNA is isolated from each sample by cell lysis using the phenol chloroform extraction method. The primers are designed particularly for GSTP1 gene from the website of NCBI through primer BLAST. The PCR amplification is done through the template DNA with GSTP1 gene, primers (forward & reverse) and master mix. Then we obtain our amplified gene of interest GSTP1. Verification is done by taking few of it and performing agarose gel electrophoresis. Confirmation is assured by obtaining bright bands at 365 bp positions that measured by ladder DNA bands. The remaining GSTP1 from each sample is followed by restriction digestion with BsmA1 (a restriction endonuclease obtained from Bacillus Stearothermophilus A664). Then again the digested samples are performing in gel electrophoresis. After results are obtained, a statistical analysis is performed with the above results.

Conclusion: From our study we conclude that prostate cancers have been found to be associated more with the G alleles or substitution of valine from isoleucine in the GSTP1 gene.

Keywords: GSTP1 Gene; Xenobiotic metabolism; Phenol chloroform; Electrophoresis; Isoleucine

Introduction

Glutathione S-transferases (GSTs) (EC 2.5.1.18) are a family of enzymes that play an important role in detoxification by catalysing the conjugation of many hydrophobic and electrophilic compounds (eg. Xenobiotic drugs, toxins, carcinogens) with reduced glutathione. GSTs are believed to play a role in protection of cells from toxicities of xenobiotic compounds as well as lipid hydro peroxides generated by oxidative stress. Mammalian GSTs are classified into nine distinct gene families: 7 cytosolic groups, 1 microsomal and 1 present in erythrocyte [1].

Prostate cancer is the development of cancer in prostate gland in male reproductive system. Most prostate cancer is slow growing. Factors that increase the risk are older age, family history, etc. In 99% of males those are affected they are generally over the age of 50. In US it is more seen in African-American population than in white population .Mortality of prostate cancer depends on the higher serum prostate specific antigen (PSA) level.

Initiation of cancer is attributable to several genetic disarrangements including chromosomal deletion, translocation, changes in DNA methylation and point mutations. These genetic changes become particularly important when they affect the expression of tumor suppressor proteins. Glutathione-S-Transferases (GSTs) belong to one of such tumor suppressor proteins which restrict the initiation and progression of tumor genesis by detoxifying different toxic carcinogens and reactive oxygen species (ROS). It also infers that when the mutations occurs at specific positions and for bases which codes for the mRNA of GSTs, then the functioning of GSTP1 gets disrupted and chances of cancer gets increased [2].

The GSTP1 gene is approximately 4 kb in length, comprises 7 exons and 6 introns and codes for a 715 base mRNA. GSTs have several ribozymes with almost similar functions in different tissues. They are responsible for metabolism and biosynthesis of various metabolites including detoxification of exogenous carcinogen chemicals like polycyclic aromatic hydrocarbon which are abundant in diesel fuels, cigarette smoke and grilled meats. Overall, they detoxify several carcinogenic xenobiotic by conjugation with glutathione during the phase II of detoxification process of the electrophonic carcinogenic compounds. GSTs have been subdivided into eight classes designated Alpha, Mu, Pi, Theta, Zeta, Kappa, Sigma, and Omega. Specific GST isoforms in the Alpha1 (A1), Mu1 (M1), and Theta1 (T1) and Pi1 (P1) classes are highly expressed in the prostate tissues. Among the large family of their ribozymes, the P1 class of enzyme which is GSTP1 is well studied in different types of cancers. In the genetic polymorphic profile of GSTP1 may be associated with severity as well as the risk of recurrence of prostate cancer which has been strongly suggested by the present research works that found significant link between GSTP1 polymorphism and need for a repeat biopsy to evaluate a progression of prostate cancer [3].

GSTP1 is mainly expressed in the basal layer of normal prostate epithelium. Its expression has been found to be significantly down-

*Corresponding author: Soujanya Ghosh, Biotechnologist and clinical researcher, Haldia Institute of Technology, Haldia, India, E-mail: soujanyaghosh.nature@gmail.com

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regulated in the initial stages of majority of adenocarcinoma including the prostate cancer. The potential GSTP1 gene promoter site remains unmethylated of an "A" base at the 303 position. Another important SNP in the GSTP1 gene was found to be Ile105Val (A to G) that replaced isoleucine by valine at the 105th position of the GSTP1 protein causing significant reduction in the detoxifying capability of this GSTP1 isoenzyme.

Interaction of GSTP1 with oxidative stress, drugs, inflammatory mediators and allergic reactions GSTP1 expression is strongly influenced by oxidative stress as a defence mechanism through the binding of transcription factors Nrf-2 and activator protein (AP) to the antioxidant response. Reverse is also true oxidative stress can inactivate GSTP1 by intermolecular disulphide formation (Oligomerization). Several electrophilic agents induce an irreversible crosslinking of the enzyme. The components that are involved in the modification are most reactive cysteines. Both GSTP1 oligomerization and crosslinking affect its interactions with signaling molecules and stress cascades. GSTP1 displays multiple interactions with drugs, either catalysing their detoxification by GSH (S-Glutathionylation) conjugation or being inactivated by them. These interactions are crucial for cancer therapy [4] and (Figure 1).

Materials & Methods

Subject and specimen collection

Patients diagnosed with adenocarcinoma of the prostate gland on the basis of clinical investigations, histopathology and prostate specific antigen were selected. At first, cases were selected provisionally on the basis of clinical investigation. As PSA is specific for prostate tissue and not for prostate cancer only, there is a considerable overlapping of the PSA values between BHP and CA prostate. So, it is difficult to assign a PSA value with 100% sensitivity and specificity for CA prostate only.

This was a hospital-based case-control study. Case-control studies are often used to identify factors that may contribute to a medical condition by comparing subjects who have that condition/disease (the "cases") with patients who do not have the condition/disease but are otherwise similar (the "controls"). Here, the control is that human gene of GSTP1 where mutation has not occurred and prostate cancer is not diagnosed. These will be the basis of comparison to others samples.

Enzymes used in this experiment

Restriction endonuclease: The restriction enzyme used to determine the SNPs (single nucleotide polymorphism) here is BsmA1. It is a unique restriction endonuclease, which is extracted from the organism Bacillus Stearothermophilus. The cleavage site was first determined from bacteria by the method of Brown et al. Incubation temperature is 55°C.

Recognition site is:

5' GTCTC 3'

3' CAGAG 5'

Cleavage site is:

G T C T C N/N N N N
C A G A G N N N N N/

It is type 1 restriction enzyme.

Isochizomers-Pairs of restriction enzymes specific to the same recognition sequence.

Isochizomers of BsmA1 are Alw261, BcoDI [5].

Taq polymerase: This is a kind of DNA polymerase that used for PCR which is extracted from the organism *Thermus Aquaticus*. This DNA polymerase can tolerate and act in high temperature (upto approx 95°C). It can extend a new DNA strand at temperature of 72°C by template strands in PCR cycle to amplify the gene of interest (Figure 2).

PCR optimization

- Perfect thermal cycling conditions.

- Specificity depends on the choice of primers and Mg2 + concentration.

Primer Designing

The basic guidelines are:

- Self-complementary primers were avoided. Especially complementary more than 3 bp should not be present or else it will form a hairpin loop.

- Primers were chosen that are specific to the target. Simple sequence repeats or commonly repeated sequences were avoided. If the target has close relatives the primers should be designed in such a way that it anneals to the target site only.
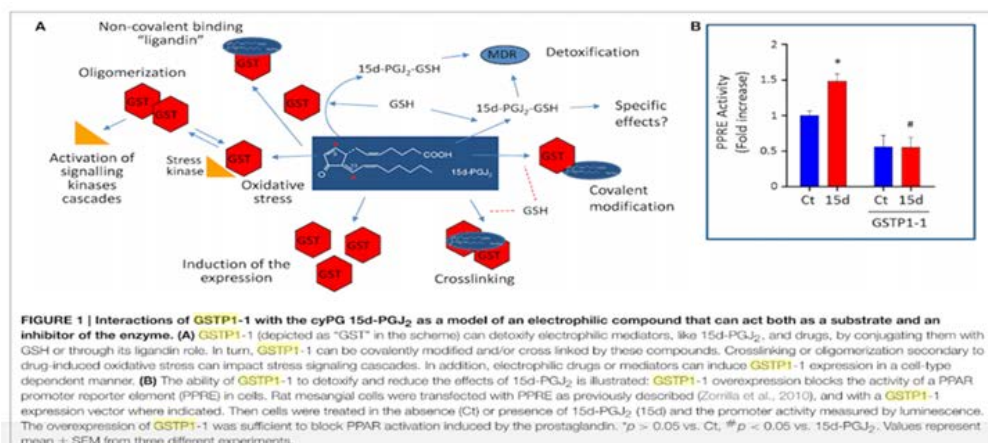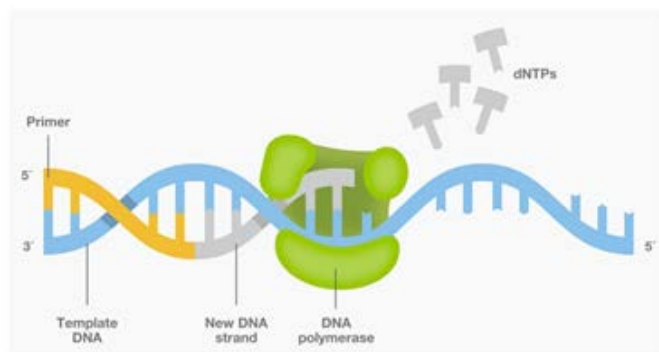

FIGURE 1 | Interactions of GSTP1-1 with the cyPG 15d-PGJ₂ as a model of an electrophilic compound that can act both as a substrate and an inhibitor of the enzyme. (A) GSTP1-1 (depicted as "GST" in the scheme) can detoxify electrophilic mediators, like 15d-PGJ₂, and drugs, by conjugating them with GSH or through its ligandin role. In turn, GSTP1-1 can be covalently modified and/or cross linked by these compounds. Crosslinking or oligomerization secondary to drug-induced oxidative stress can impact stress signaling cascades. In addition, electrophilic drugs or mediators can induce GSTP1-1 oligomerization in a cell-type dependent manner. (B) The ability of GSTP1-1 to detoxify and reduce the effects of 15d-PGJ₂ is illustrated: GSTP1-1 overexpression blocks the activity of a PPAR promoter reporter element (PPRE) in cells. Rat mesangial cells were transfected with PPRE as previously described (Zorrilla et al., 2010), and with a GSTP1-1 expression vector where indicated. Then cells were treated in the absence (Ct) or presence of 15d-PGJ₂ (15d) and the promoter activity measured by luminescence. The overexpression of GSTP1-1 was sufficient to block PPAR activation induced by the prostaglandin. *p > 0.05 vs. Ct, #p < 0.05 vs. 15d-PGJ₂. Values represent mean ± SEM from three different experiments.

Figure 1: Interaction of GSTP1 with different compounds.

Figure 2: Taq polymerase mechanism.

• Primers between 18 – 25 bp were used that have matched melting temperature (Tm) to each other. A primer greater than 17 bp has a good chance of being unique in the human genome.

• It was better to avoid amplifying longer targets, product length less than 500 bases were recommended. Shorter products amplify with higher efficiency.

• Complementary between members of primer pairs should be avoided. The 3' complementary is detrimental. Primer dimer will compete for DNA polymerase, primers and dNTPs which will suppress amplification.

• Depending on the purpose of the experiment the placement of the priming sites should be taken into consideration. Generally forward and reverse primers bind to sequences in different exons [6].

Positive and Negative control for PCR

Laboratories using PCR should analyze positive and negative quality control samples on a routine basis to demonstrate the adequate performance of PCR-based methods.

Positive controls were used to verify that the method was capable of amplifying the target nucleic acid from the organism of interest. In this, a known sample was given with the same master mix and dNTPs to see if it was properly amplified.

A negative control was used to verify that no contaminating nucleic acid had been introduced into the master mix, distilled water, and dNTPs. In this only master mix, dNTPs and water are given and PCR cycle is performed along with our sample.

A negative control was one expected not to work under the conditions.

A positive control was one expected to work and to provide you with the expected known result.

Methods in the specific order

Genomic DNA extraction: Blood was taken in sufficient amount. Organic (phenol–chloroform) extraction uses sodium dodecyl sulphate (SDS) and protease for cell lysis by the enzymatic digestion of proteins and non-nucleic acid cellular components. A mixture of phenol: chloroform: isoamyl alcohol (25:24:1) was then added to promote the partitioning of lipids and cellular debris into the organic phase and breaking RNA by adding RNase, leaving isolated DNA in the aqueous phase. Following centrifugation, the aqueous phase containing the purified DNA can be transferred to a clean tube for analysis. After

isolation, the DNA was dissolved in slightly alkaline buffer, usually in the TE buffer [7].

Checking the viability of DNA:

• Using spectrophotometer –It was used to check the amount of DNA. First the OD was measured at 260 nm for DNA. Then OD was measured at 280 nm for protein. Ratio between the two was taken i.e. OD260/OD280 > 1.8 (Figure 3).

• Using gel electrophoresis- It was used to check the integrity of the DNA. If weak DNA is obtained we will get smeared DNA after gel electrophoresis. If DNA is normal we will get bands at specific position. Prepared 0.7% agarose gel.
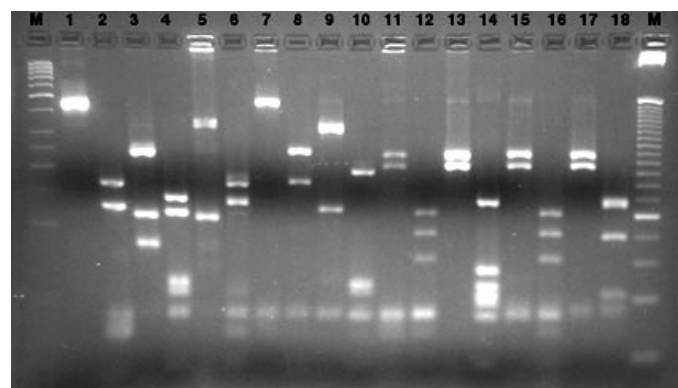
30 ml TEA buffer+ 0.21 g agarose was taken and mixed properly.

Then microwave for 1 min to dissolve it properly. EtBr was then added to visualize in UV ray. Leave sometimes to cool a bit. Put it in the gel tray, insert the comb and allow solidifying. Put it in the chamber, submerge it with TEA running buffer and insert the DNA samples with the tracking dye (Figure 4).
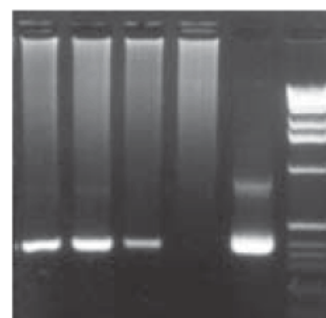
PCR amplification of GSTP1: For PCR amplification four things are required-

i. Primer (Forward & reverse)

ii. Master mix (MgCl2 , buffer, dNTPs, Taq polymerase)

iii. DNA Template

iv. Deionised & nuclease free double distilled water.

Primers were designed accordingly from NCBI primer BLAST. The forward and reverse primers selected were 5′-GTCTCTCATCCTTCCACGCA-3′ and

Figure3: DNA bands.

Figure4: Smeared DNA.

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5'-CTGCACCCTGACCCAAGAA-3' respectively.As per the protocol each PCR tube should be of 25µl filled. 12.5µl Master mix, 2µl primers, 1µl DNA template, 9.5µl distilled water are added in each PCR tube. The PCR should be programmed as follows-

i.   Pre-heating- 95°C for 2mins   -For 1 time

ii.   Denaturation- 95°C for 30 secs

iii. Annealing- 60°C for 30 secs   For 30 cycles

iv.   Extension- 72°C for 1 min

v.   Extended phase- 72°C for 5 mins   -For 1 time

The PCR process is performed in gradient PCR obtained from Applied Biosystems.

A part of PCR products obtained were run in 2% agarose gel (0.6 g agarose) against 100 bp DNA ladder and were identified at 365 bp using the Gel Doc system or E gel Imager obtained from Applied Biosystems for time of 1 hr 22 mins.

For each DNA sample about 10 µl PCR sample and 8 µl tracking dye is given. Then loaded those samples and 100 bp ladder (3-5µl) in the wells and run electrophoresis.

The GSTP1 gene will produce a bright band at 365 bp which will be between 300 bp and 400 bp of the ladder (Figure 5).

Restriction digestion: PCR products those are perfectly obtained were digested using the restriction enzyme BsmA1. The things required for the digestion are for each PCR sample is:

i.   Buffer (Tris HCl) 10x   2.5 µl

ii.   Restriction enzyme(BsmA1) 1.5 µl

iii. PCR DNA   12 µl

iv.   Distilled Water   9 or 10 µl

Incubate for 2-3 hours at 37° to 40°C into hot water bath for the restriction digestion. After the restriction digestion has occurred, gel electrophoresis is done in 2.5% agarose gel i.e. 30 ml TEA buffer and
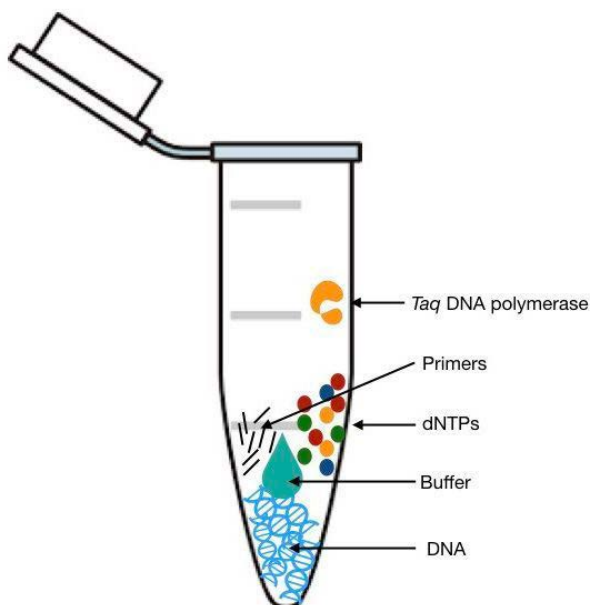
0.75 g agarose. We will obtain either 1 band or 2 band or 3 bands in each lane. It will define the type of polymorphism that had occurred. When compared against 100 bp DNA ladder, the PCR product of GSTP1 gene was reflected by the undigested wild AA homozygous genotype and was found to be of 365 bp as expected for the given set of primers. The mutant GG homozygous genotype showed two digested products of 140 bp and 225 bp. On the other hand, the mutant AG heterozygous genotype showed three bands of 365, 225 and 140 bp.

- **Cases Performed According to Above Methods**

➢ **Case I**

i.   Some genomic DNA samples of any prostate adenocarcinoma positive patients are selected which are mostly viable by spectrophotometry and 0.7% agarose gel run. Those sample number was 5, 7, 32, 34, 35, 36, and 37.

ii.   For these 7 samples we took the master mix, primers and nuclease free distilled water for amount of 8 samples. Those are equally distributed in each PCR tube of 7 samples (1µl of each sample) and make total volume 25µl of each tube as above methods. 7 samples were set to run in gradient PCR for 30 cycles with same programming.

iii. After PCR completed, loading dye was added in each of 10µl sample. Centrifuge all samples by 6000 rpm for 1 min. Then samples were run to electrophoresis by 2% agarose gel by putting samples in wells (lane 1- ladder, lane 2- 5th, lane 3- 7th, lane 4- 32nd, lane 5- 34th, lane 6- 35th, lane 7- 36th, lane 8- 37th).

After running we saw in gel doc system that 5th & 7th sample not amplified and bands not occurred. 32, 34, 35, 36, 37 samples able to occur clear band at 365 bp measured by ladder (Figure 6).

iv.   Then for those 5 samples were taken for restriction digestion. The amount of BsmA1, distilled water, 10x buffers was taken for total 5 samples. This mixture was equally distributed in rest of each samples as per above protocol and made the total volume of 25 µl each. Then incubate it for approx 2 hrs. After incubation, those digested 5 samples were allow to run in electrophoresis by 2.5% agarose gel after adding tracking dye into the samples (lane 1- ladder, lane 2- 37th, lane 3- 35th, lane 4- 34th, lane 5- 32nd, lane 6- 36th).

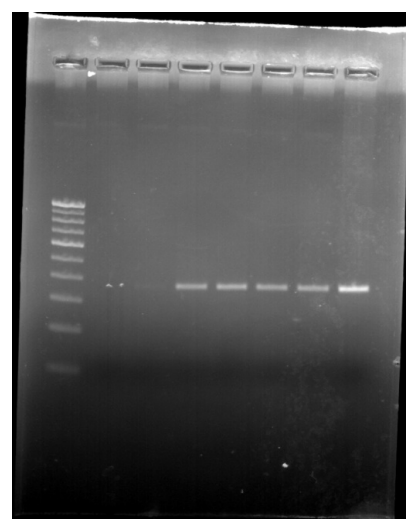After running we saw in gel doc system that 37th (lane 2), 35th (lane

Figure 5: PCR mix.

Figure 6: Lane 2, 3- not amplified. Lane 4, 5, 6, 7, 8- amplified & gives band at 365 bp Lane 1- 100 bp ladder.

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3) and 32nd (lane 5) samples were uncut and sharp band occurred at 365 bp position. Sample number 34th (lane 4) & 36th (lane 6) was cut into 3 bands at 365 bp, 225 bp, 140 bp positions measured by 100 bp ladder (Figure 7).

➤ **Case II**

i. Some prostate adenocarcinoma positive DNA samples were taken and those sample numbers are 51, 52, 54, 55, 57, 58, 59, 61, 62, 64, 66, 82, 83 & 84. Then we check the amount of DNA of those samples by spectrophotometer and check the integrity of DNA of those samples through electrophoresis by 0.7% agarose gel. We saw by gel doc system that 57th sample give a very week band and 64th, 66th & 84th samples give bright and strong band. So we took 57,64,66,84 number samples for PCR.
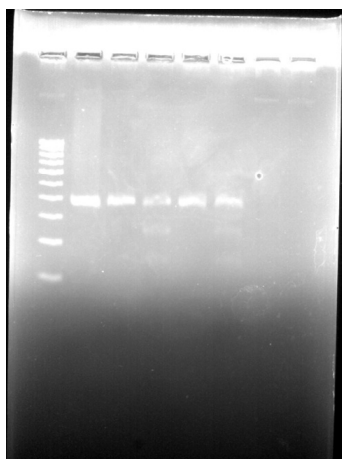
ii. Then create a master mix as per above protocol by taking amount for one extra sample means for 5 samples. In each PCR tube, 24 μl master mix and 1.2 μl selected DNA samples were distributed. These 4 samples were set to run in gradient PCR for 30 cycles with same programming.

iii. After PCR completed, loading dye was added in each of 10μl sample. Centrifuge all samples by 6000 rpm for 1 min. Then samples were run to electrophoresis by 2% agarose gel by putting samples in wells (lane 1- ladder, lane 2- 57th, lane 3- 64th, lane 4- 66th, lane 5-84th).

After running we saw in gel doc system that 57th & 84th sample not amplified and bands not occurred. 64 & 66 samples able to occur clear band at 365 bp measured by ladder.

iv. Then we took the previous DNA samples 35th & 37th which were uncut after restriction digestion. So we ready more 3 PCR tubes with these 2 samples as 35th, 37th, & 37th duplicate (37D). Performed in gradient PCR for 30 cycles with same programming. After PCR completed, loading dye was added in each of 10μl sample. Centrifuge all samples by 6000 rpm for 1 min. Then samples were run to electrophoresis by 2% agarose gel by putting samples in wells along with 64th & 66th samples.

After running we saw in gel doc system that every sample gave a clear band at 365 bp position (Figure 8). But as 35th & 37th sample was performed upto digestion previously so that we didn't take 35, 37, 37D samples now for digestion. We took 64th & 66th samples for digestion.

v. For restriction digestion, the amount of BsmA1, distilled water, 10x buffers was taken for total 2 samples. This mixture was equally distributed in rest of each samples as per above protocol and made the total volume of 25 μl each. Then incubate it for approx. 2 hrs. After incubation, those digested 2 samples were allow to run in electrophoresis by 2.5% agarose gel after adding tracking dye into the samples (lane 1- ladder, lane 2- 64th, lane 3- 66th).

After running we saw in gel doc system that both samples remain uncut and gave a clear band at 365 bp position (Figure 9).
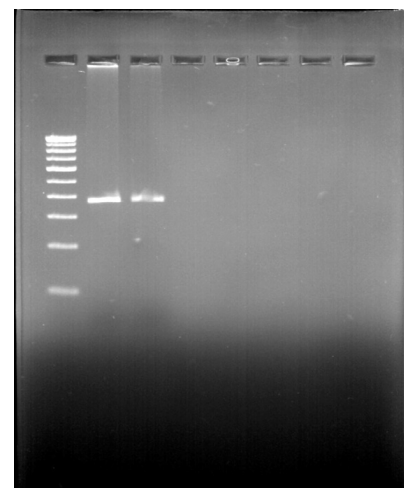
➤ **Case III (control)**

i. Some prostate adenocarcinoma negative DNA samples (un-mutated DNA) were taken and samples are 2, 4, 10, 16, 24, DNA control (C). We check the integrity of DNA of those samples through electrophoresis by 0.7% agarose gel. We saw by gel doc system that sample number 2 (lane 2) & 24 (lane 6) gave very weak band and sample number 4, 10, 16, C (lane 3,4,5,7 respectively) gave very strong band (Figure 10). So we took 4, 10, 16, C samples for PCR.
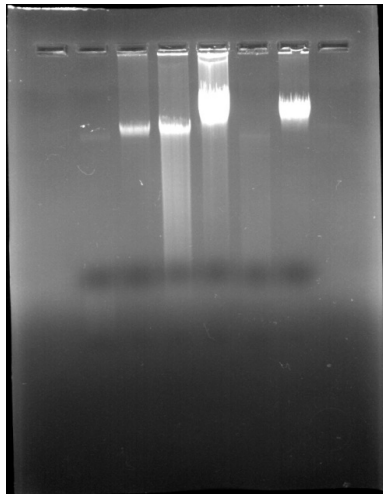
ii. Then create a master mix as per above protocol by taking amount for one extra sample means for 5 samples. In each PCR tube, 24 μl master mix and 1.2 μl selected DNA samples were distributed.
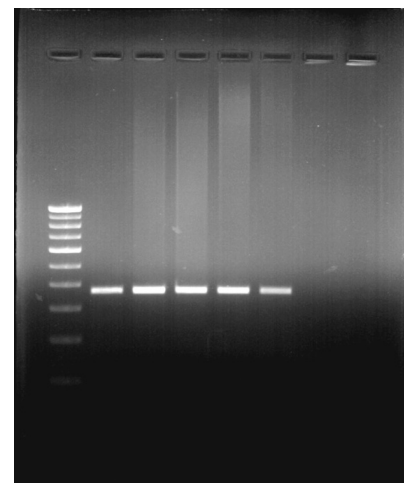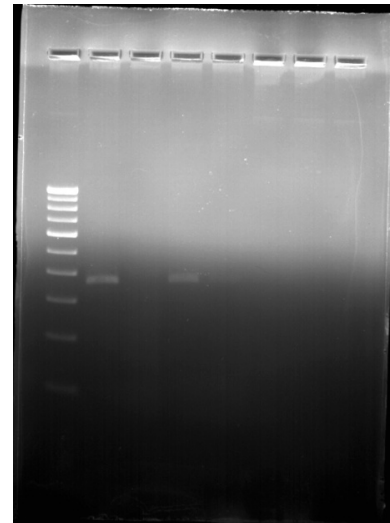

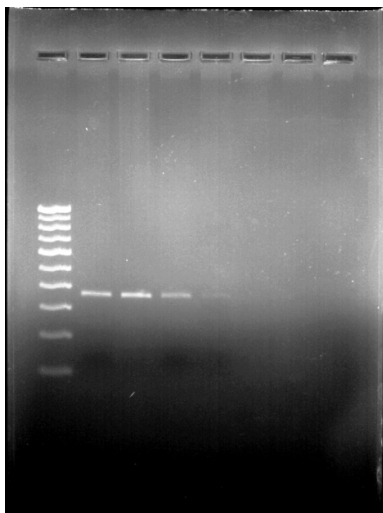**Figure 8:** Lane 2, 3, 4, 5, 6- amplified & gives band at 365 bp. Lane 1- 100 bp ladder.

Figure 7: Lane 2, 3, 5- uncut AA homozygous. Lane 4, 6- cut and bands at 365,225,140 bp causes AG heterozygous. Lane 1-100 bp ladder.

Figure 9: Lane 2, 3- uncut AA homozygous Lane 1- 100 bp ladder.

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Figure10: Lane 2, 6- gave weak band. Lane 3, 4, 5, 7- gave strong band.

Figure 12: Lane 2, 4- uncut AA homozygous. Lane 3- cut and smear means GG homozygous. Lane 5- uncut and give so much weak band means AA homozygous causes its PCR product was very weak. Lane 1- 100 bp ladder.

Figure 11: Lane 2, 3, 4, 5- amplified & give band at 365 bp but lane 5 give very weak band Lane 1- 100 bp ladder.
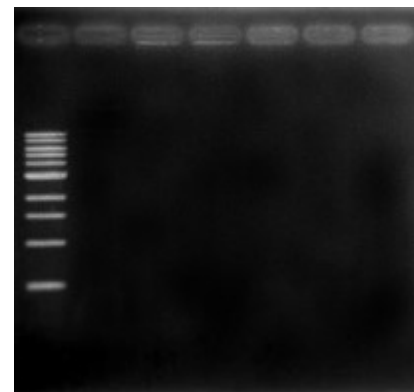

Figure 13: PCR product not amplified during 1st time case II Lane 1- 100 bp ladder.

These 4 samples were set to run in gradient PCR for 30 cycles with same programming.

iii. After PCR completed, loading dye was added in each of 10μl sample. Centrifuge all samples by 6000 rpm for 1 min. Then samples were run to electrophoresis by 2% agarose gel by putting samples in wells (lane 1- ladder, lane 2- 4th, lane 3- 10th, lane 4- 16th, lane 5- C).

After running we saw in gel doc system that all samples were amplified and 4th, 10th, 16th samples gave clear sharp band and sample C gave very weak band and all are at the 365 bp position (Figure 11). So we took all samples for digestion.

iv. For restriction digestion, the amount of BsmA1, distilled water, 10x buffers was taken for total 4 samples. This mixture was equally distributed in rest of each samples as per above protocol and made the total volume of 25 μl each. Then incubate it for approx 3 hrs. After incubation, those digested 4 samples were allow to run in electrophoresis by 2.5% agarose gel after adding tracking dye into the samples (lane 1- ladder, lane 2- 4th, lane 3- 10th, lane 4- 16th, lane 5- C).

After running we saw in gel doc system that sample number 4 &

16 remains uncut and give a clear band at 365 bp position and sample C also uncut and give a so much mild band at 365 bp position. But the sample number 10 didn't give any band and get smeared may cause of over digestion (Figure 12).

Note that:- In case II we repeated the PCR stage for 2nd time because in 1st time the PCR products were not amplified and not come any bands while viewing through gel doc system causes any kind of manual errors (Figure 13).

Statistical analysis

The results are represented on pie chart (Figure 14) -

Comparison of the distribution of different GSTP1 alleles between the case and control groups is performed by chi square test and odds ratio analysis.

Chi Square test:

Case (n=7) & Control (n=4)

Due to very small sample size chi square test cannot be performed (Table 1).
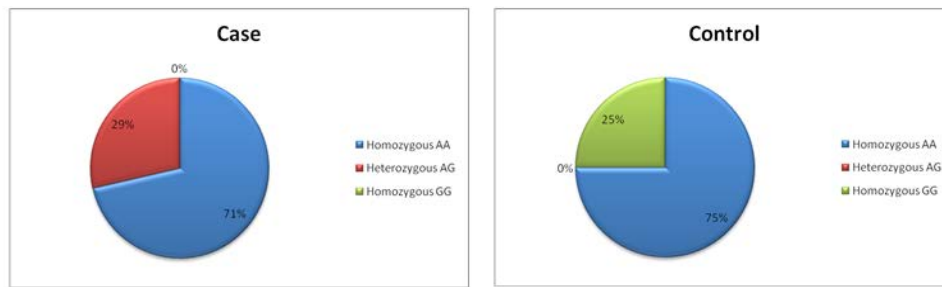
Odds Ratio:

Figure 14: Comparison of the distribution of different GSTP1 alleles between the case and control groups is performed by chi square test and odds ratio analysis.

Table 1: Chi Square test.

	Homozygous AA	Heterozygous AG	Homozygous GG	Chi Square	P value
Case	5	2	0		Not performed
Control	3	0	1	-	

Table 2: Odds Ratio.

	A alleles	G alleles
Case	12	2
Control	6	2

Now,

O.R. = 0.5, Range = 0.056 to 4.474 at 95% confidence interval (Table 2).

Discussion

By this project we used to found that how many types of genotypes or alleles may occur by SNPs which are the main cause of prostate adenocarcinoma.

We saw that AG and GG alleles of GSTP1 gene polymorphisms are considered as risk for prostate cancer. In case I digestion we obtain AG genotype of polymorphism; in case II digestion we don't obtain any polymorphic genotype; but in case III of control sample digestion we unfortunately obtain GG genotype of polymorphism that instead of control sample, it also very near to the case samples which are positive in prostate adenocarcinoma.

Chi-square test cannot be performed due to low data samples. It is also true that the chi-square test is performed only if at least 80% of the cells have an expected frequency of 5 or greater, and no cell has an expected frequency smaller than 1.0.

A significantly higher association of prostate cancer with the G allele in GSTP1 gene is found by the odds ratio of 0.5 with a 95% confidence interval of 0.056 to 4.474. This means that the chances of having prostate cancer with G allele are 0.5 times more than with association to an allele. However, a major drawback of this result is due to extremely small sample size.

GSTP1 gene polymorphisms may be considered as factors increasing the susceptibility to and risk of Hepatocellular carcinoma (HCC). HCC is one of the most frequent malignant neoplasms in the world. Genetic polymorphism has been reported to be a factor increasing the risk of HCC [8].

Expression of GSTP1 is regulated mainly at the transcriptional level. It has been suggested that replacement of isoleucine with the less bulkier but more hydrophobic valine in the protein results in the alteration in substrate binding capability of its catalytic site and hence reduction in its detoxifying capability of the pro oxidant heterocyclic amine carcinogens [9].

GSTP1 polymorphism is significantly associated with risk of prostate cancer. GSTP1 polymorphism should be considered as a prognostic indicator for prostate cancer patients [10].

We have observed the polymorphism in the GSTP1 gene. Polymorphisms are the low-penetrable mutations that make us all so subtlety different. It is estimated that up to 85% of such theoretical SNPs cannot be confirmed in population statistic studies but in this project, we are already aware of the population statistics from the literature available. GSTP1 polymorphisms are important in mediating clinical phenotypes for cancers including prostate cancer. We have undergone through the protocol to prove the relation between GSTP1 polymorphism and prostate cancer and we have learned the processes involved, to perform the experiment.

Conclusion

From our study, we conclude that prostate cancers have been found to be associated more with the G alleles or substitution of valine from isoleucine in the GSTP1 gene. It is also true that GSTP1 polymorphism of A to G is significantly associated with the risk of prostate cancer. Thus, it should be considered as an important biomarker for detecting prostate cancer.

References

1. GSTP1 Wikipedia, the free encyclopedia.

2. Roy S, Dasgupta A, Chatterji S, Karmakar D (2019) Correlation of GSTP1 Polymorphism with Severity of Prostate Cancer in an Eastern Indian Population. J Adv Med 29: 1-10.

3. Glutathione S-transferase Wikipedia, the free encyclopedia.

4. Sánchez-Gómez FJ, Díez-Dacal B, García-Martín E, Agúndez JA, Pajares MA, et al. (2016) Detoxifying Enzymes at the Cross-Roads of Inflammation, Oxidative Stress, and Drug Hypersensitivity: Role of Glutathione Transferase P1-1 and Aldose Reductase. Front Pharmacol 7: 237.

5. BsmAI Enzyme Finder, New England Biolabs.

6. Purich DL, Allison RD (2002) The Enzyme Reference. San Diego, California: Elsevier Science 134.

7. Sambrook J, Russel DW (2001) Molecular cloning: A laboratory manual. New York: Cold spring harbour laboratory press.

8. Phenol-Chloroform Extraction.

9. Yao-Li C, Hsin-Shun T, Wu-Hsien K, Shun-Fa Y, Dar-Ren C, et al. (2010)

Glutathione S-Transferase P1 (GSTP1) gene polymorphism increases age-related susceptibility to hepatocellular carcinoma. BMC Medical Genetics 11: 46.

10. Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, et al. (1998) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19: 433-436.