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# Genetic Polymorphisms in Estrogen Metabolizing Gene Pathway and Ovarian Cancer Risk

**ANDREA MARY F[1], JUDES NISHA A[2], ANITHA RAMESH[3], SOLOMON F D PAUL[4]**

[1]Dept of Human Genetics, Faculty of Biomedical Sciences, Research and Technology , Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India, E-mail: jamant_f@yahoo.co.in.

[2]Dept of Human Genetics, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India, E-mail: nisha.alexander07@gmail.com

[3]Professor & Head, Dept of Oncology, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India.

[4]Professor & Head, Dept of Human Genetics, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India, E-mail: wise_soly@yahoo.com.

**Abstract:** Ovarian cancer is the leading cause of cancer death in women. The prevalence of ovarian cancer is 19.8% in India. Genetic polymorphisms in steroid metabolizing enzymes may play an important role in the susceptibility of individuals to ovarian cancer. Polymorphisms of the CYP1B1 and SULT1A1 genes that encode for the enzymes that metabolize estrogen are linked to hormone-related cancers. Studies are limited on this pathway in ovarian cancer. In the present study genetic polymorphism in CYP1B1 and SULT1B1 gene were evaluated, in 20 controls and 20 ovarian cancer affected cases. Subjects were genotyped for two common single nucleotide polymorphisms in two genes involved in catechol estrogen formation (CYP1B1) or conjugation (SULT1A1) denoted as V432L and G638C by PCR-Restriction fragment length polymorphism with the enzyme Acu1 (CYP1B1) and Hha1 (SULT1A1). Among the cases the C/C genotype for rs1056836 was much higher than in the controls suggesting that the Val432Leu polymorphism of the CYP1B1 gene may represent as a possible genetic risk factor. One case sample for SULT1A1 showed heterozygous genotype (G/A). Representative samples were further confirmed by DNA sequencing. These findings have potentially important implications for genetic risk assessment and it is the first study on polymorphisms of steroid metabolizing enzymes in ovarian cancer. Studies with large number of samples are needed in order to define genetic ovarian cancer risk factors.

**Keywords:** Ovarian Cancer, Polymorphism, Estrogen, Sequencing, CYP1B1, SULT1A1.

## I. INTRODUCTION

Ovarian cancer is one of the most deadly cancers with highest mortality rates it is the 11th most common and 5th leading cause of cancer related death in women. (Cannistra SA 2004) It accounts for about 3% of cancer in women (American cancer society 2008; Runnebaum IB 2001). The prevalence rate of ovarian cancer is about 19.8% (Ramnath Takiar 2010). It has been put forward that ovulation increases the risk of ovarian cancer development due to mutation in the epithelium at the ovulatory site which may stimulate the production of sex steroid hormone, which in addition enhances the transformation and proliferation in the ovarian epithelium. Thus, the polymorphisms in the genes which regulate these processes, such as genes involved in the pathway of sex steroid hormone metabolism and biosynthesis could influence the ovarian cancer susceptibility (Fasching etal., 2009; Holt SK Peter 2007 and Pike 2004). Sulphotransferase (SULT1A1) gene catalyses the conjugation of sulfate groups located in chromosome 16p11.2 - p12.1 15 (Blanchard RL 2004) SULT1A1 plays an important role in the phase II metabolism of a large number of exogenous endogenous compounds ( Liang G 2004).

In the exon 7 of the SULT1A1 gene a common polymorphism denoted as G638C has been reported that results in the substitution of a histidine in place of arginine at 213 position of the translated protein, which produces an enzyme with decreased catalytic activity thermal stability (Aftogianis RB 1999). The CYP1B1 gene contains three exons involved in phase 1 metabolism of xenobiotics. The polymorphism Val432Leu allele of the CYP1B1 gene, associated with high-activity of the enzyme produces elevated 4-hydroxylated catecholestrogen formation, linked to oxidative stress with an increased risk of ovarian cancer (Cavalieri E et al., 1997, Good man MT et al., 2008). Polymorphisms in genes that regulate the concentration of estrogens and their metabolites may contribute directly to the individual variation in ovarian cancer risk through a mechanism involving oxidative stress or indirectly by influencing ovarian cancer susceptibility associated with ovulation and reproduction (Laetitia Delort et al., 2008). The estrogen-signaling metabolic pathway plays a pivotal role in the pathogenesis of ovarian cancer; therefore, polymorphism in the genes involved in estrogen signaling pathway is likely to influence ovarian cancer risk.

## II. MATERIALS AND METHODS

A formalized informed consent form was used to procure approval from the subjects to proceed with the sampling.

High molecular weight genomic DNA was successfully isolated from 5ml of peripheral blood samples in EDTA vials from 20 ovarian cancer affected subjects and 20 age matched control samples by high salting out method. The participants age ranged from 25-67yrs. The exon 7 of SULT1A1 was amplified by using specific primers 5'-TCCAGAATCT GTTCCAGAGCGTGC-3'(Forward) and 5'- CTTGGGGAG AACCATCCTCA -3' (Reverse) and and exon 3 of CYP1B1 was amplified by using specific primers Forward Primer - 5'-TCACTTGCTTTTCTCTCTCC- 3' Reverse Primer - 5'-AATTTCAGCTTGCCTCCTG-3' for CYP1B1. The cycling conditions for SULT1A1 were 94º C for 5min of one cycle; 94º C for 30sec, 58º C for 30sec and 72º C for 45sec for 30 cycles and final elongation cycle of 72ºC for 5min ( Kalyan Kumar Ch et al., 2013).The cycling condition for CYP1B1 was 95º C for 5min of one cycle; 94º C for 30sec, 60.8º C for 30sec and 72º C for 45sec for 30 cycles and final elongation cycle of 72ºC for 5min. The PCR amplicon of 200bp (Fig 2) was subjected to restriction digestion using Hha1 enzyme (New England Biolabs) for SULT1A1( Kalyan Kumar et al., 2013) and the PCR amplicon of 650bp of CYP1B1(Fig1) was subjected to restriction digestion with Acu1 (Fermentas) for CYP1B1 at 37°C for 1hour (Wei Zheng 2001). The DNA bands were resolved by electrophoresis on a 3% agarose gel. The genotypes were determined based on the band pattern (Fig:3,4). Representation samples were confirmed using DNA sequencing. (Fig:5,6,7). Allelic frequencies and Genotype distribution with those expected from Hardy-Weinberg Equilibrium (HWE) were made using chi square test, and Values of P (two -tailed) less than 0.005 were considered statistically significant(Table 1 and Table 2).

## III. RESULTS AND DISCUSSION

The present study was carried out in 20 ovarian cancer affected and 20 age matched control samples. The study was approved by ethical committee and informed consent was taken from the participants. The exon 7 and exon 3 of SULT1A1 and CYP1B1was amplified successfully with the respective primers (Fig :1,2).The PCR products are subjected to restriction digestion for the detection of V432L and G638A polymorphism and the banding pattern was interpreted (Fig3,4). The CYP1B1 results were confirmed by DNA sequencing (Figure:5,6,7). SULT1A1 is the sulfotransferase isoform plays role in the sulfation of 4-OH. Sulfation of estradiol is considered to result in the formation of inactive compound, these sulfated estrogen generally serve as poor ligands for the receptors of estrogen (Falany et al., 1997). The polymorphism G638C of SULT1A1 results in the subsituition of amino acid arginine for histine at 213 codon this base change leads to lowered activity of sulfotransferase enzyme there by lowering the deactivation potential of the enzyme. Previous investigation carried out in hormone related cancers showed a positive correlation for G638C polymorphism and cancer risk. The presence of this risk allele and the role of this enzyme (SULT1A1) in heterocyclic amine estrogen and PAH metabolism suggest that polymorphism of SULT1A1 gene may be important in the etiology of ovarian cancer.

The exon 3 of CYP1B1 codes the heme binding domain, which is crucial for the catalytic function, presence of polymorphims alter the catalytic activity towards several steroid hormones and procarcinogens (Tsuchiya 2005). CYP1B1 is the principal enzyme involved in producing the catechol estrogen. Members of the CYP1 enzyme family activate carcinogenic chemicals, including heterocyclic, polycyclic aromatic hydrocarbons and aryl amines (Nebert 2006). An association has been reported for the V432L polymorphism and risk for ovarian cancer (Goodman et al., 2001). CYP1B1 are responsible for the hydroxylation of estrogens to the 2-OH and 4-OH catechol estrogens. Estradiol and estrone are activated by Cytochrome P450-mediated pathway to 2-hydroxylated estrogen this weakly binds to the estrogen receptor and has reduced risk for ovarian cancer. The polymorphic site found in exon 3 is G •C transition in the nucleotide, which results in the substitution of valine (V) at the location 432 to leucine (L). There exists an alternate competing pathway which catalyzes the formation of 4-hydroxylated intermediate, (Yager J.D et al., 199; Zhu 1998) which is oxidized further to form 3,4 – quinones and genotoxic depurinating DNA adducts and are carcinogenic to the ovary (Adjei AA 2012).

Previous studies have shown that the risk of bone marrow cancer is enhanced by the variant allele of SULT1A1 due to decreased sulfotransferase activity (Pereira 2005). Two previous studies (Zheng 2004) reported the SULT1A1*2 polymorphism associated with ovarian cancer. Previous studies have shown inconsistent results for the association between the SULT1A1 G638A polymorphism and several malignancies including Bladder cancer, ovarian cancer and prostate cancer (Arslan 2009). Steroid hormones show a strong association with ovarian carcinogenesis but the biological mechanism of this association is not clear (Goodman et al., 2001). In the present study genetic polymorphism of the CYP1B1 and SULT1A1 gene were evaluated, with two RFLPs at the CYP1B1 and SULT1A1, denoted as Acu1 and Hha1, at the Exon 3 of CYP1B1 for the detection of V432L polymorphism and G638A polymorphism in exon 7 of SULTIA1 were examined in 20 ovarian cancer cases and 20 age matched controls. The result of V432L polymorphism in CYP1B1 gene showed higher significance in cases than controls. Genetic polymorphism analysis of the G638A in SULT1A1 gene revealed heterozygous genotype (200, 160bp length) in one of the case sample, whereas the other sample showed wild type genotype.

Similar studies reported that genotypes from case sample were significantly associated with ovarian cancer risk. This finding is consistent with some studies and deviates from others. The majority of studies suggest that genetic polymorphisms in xenobiotic metabolizing enzymes may play an important role in the susceptibility of individuals to ovarian cancer because these enzymes take part in bioavailability, detoxification, and metabolism, of steroid hormone estrogen, polymorphisms of these genes affect the metabolic activity, function and carcinogenesis (Sellers

2005). Higher activation and expression of enzymes involved in biosynthesis (CYP1B1) and lower activation and expression of conjugation enzymes (SULT1A1) may lead to increased release of toxic metabolites in system or carcinogenicity of estrogen metabolites (Hiroshi Hirata 2008).

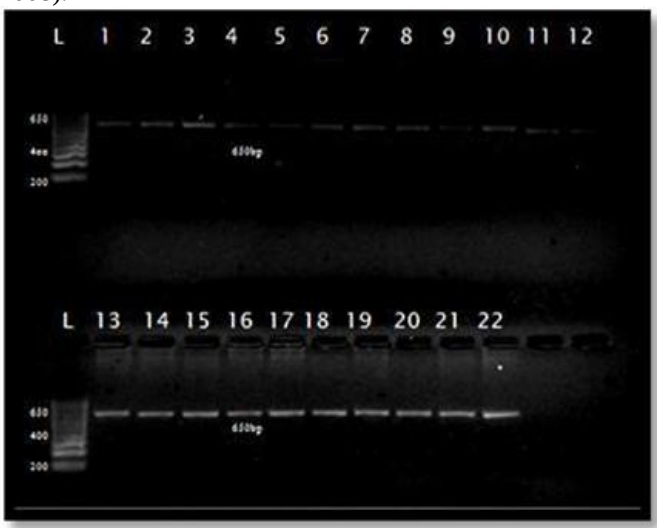


**Fig.1.Electrophoresis gel picture showing the amplification of CYP1B1 from patients sample 1-11.**

Invitro amplification of the exon 3 of CYP1BI gene corresponding to 650 base pair from the control and ovarian cancer affected individuals.

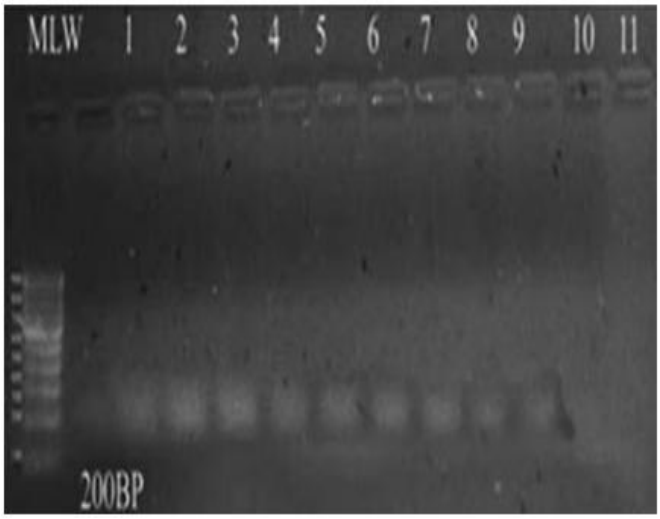


**Fig.2. Electrophoresis gel picture showing the amplification of SULT1A1 gene.**

Invitro amplification of the exon 7 of SULT1A1 gene from the control and ovarian cancer affected individuals.

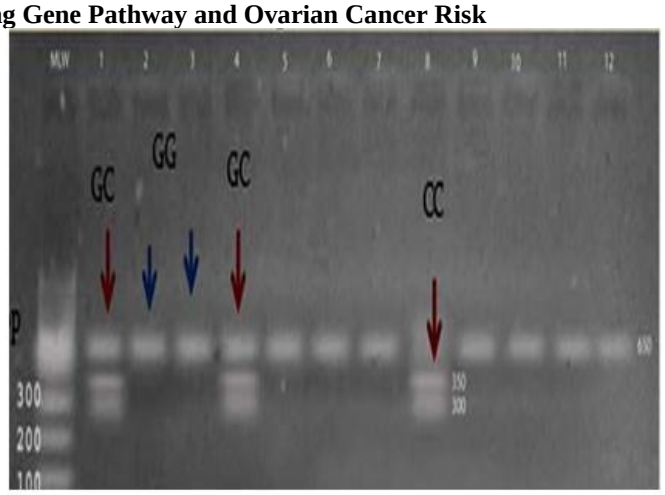


**Fig.3. CYP1B1 after restriction digestion with Acu I.**

Amplified CYP1B1 gene products on restriction digestion with Acu1 yielded homozygous wild type genotype(GG) in lane 2,3,5,6,7,9,10,11,12 corresponding to 650bp and Heterozygous genotype (GC) in Lane-1,4 corresponding to 650bp and 300 and 350 bp. Homozygous mutant genotype (CC) in lane Lane-8 corresponding to 300 and 350bp.

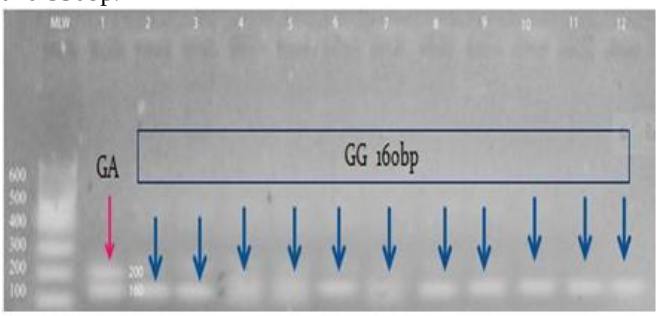


**Fig.4. SULT1A1 after restriction digestion with Hha1.**

Amplified SULT1A1 gene products on restriction digestion with Hha1 yielded homozygous wild type genotype (GG) in lane 2-12 corresponding to 160bp and 40bp. Heterozygous mutant genotype (GA) in Lane-1 corresponding to 200bp and 160bp, and 40 bp

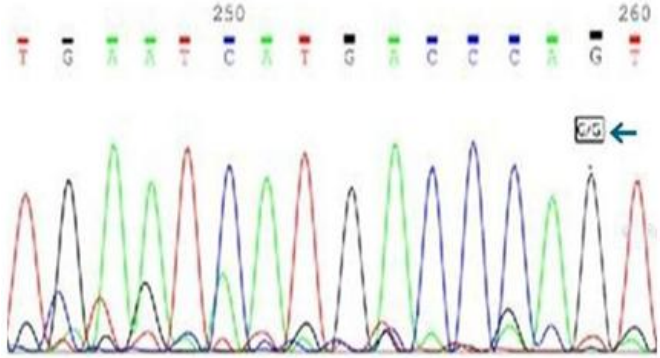


**Fig.5. Homozygous wild type genotype (GG) at nucleotide position 432 in exon 3 junction of CYP1B1 gene.**

Sequence chromatograms of CYP1B1 gene region showing Homozygous wild type genotype (GG).

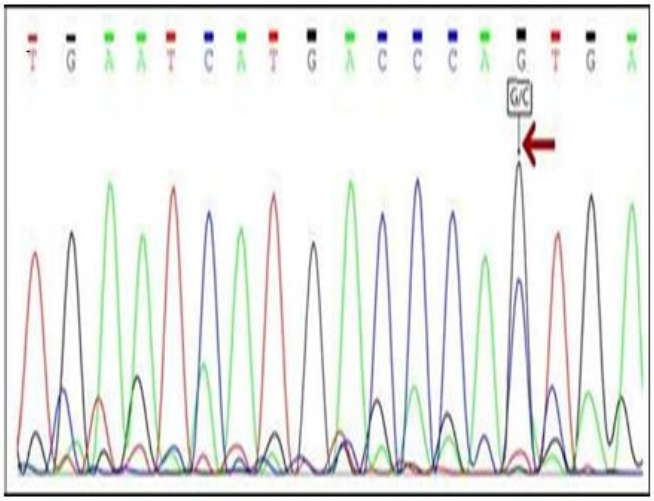

**Fig.6. Heterozygous Mutant (GC) at nucleotide position 432 in exon 3 junction of CYP1B1 gene.**

Sequence chromatograms of CYP1B1 gene region showing Heterozygous mutant type genotype (GC).

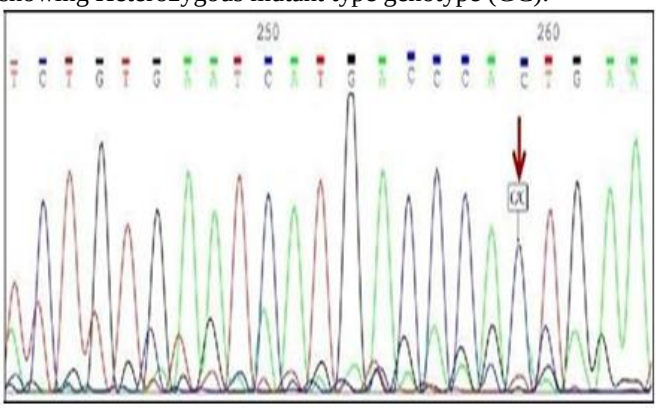

**Fig.7. Homozygyous Mutant genotype(CC) at nucleotide position 432 in exon 3 juction of CYP1B1.**

**TABLE I: Distribution of CYP1B1 Gene G432C Polymorphism in Ovarian Cancer Patients and Control**

| Genotype | Homozygous wild type (GG) | Heterozygous mutant(GC) | Homozygous mutant(CC) | Total |
|---|---|---|---|---|
| Cases | 1 | 8 | 11 | 20 |
| Controls | 15 | 4 | 1 | 20 |

The p value for the genotype CC and GC was found to be <0.002 which was significant.

**TABLE II: Distribution of SULT1A1 GENE G638A Polymorphism in Ovarian Cancer Patients And Controls**

| Genotype | Homozygous wild type (GG) | Heterozygous mutant(GA) | Homozygous mutant(AA) | Total |
|---|---|---|---|---|
| Cases | 19 | 1 | 0 | 20 |
| Controls | 20 | 0 | 0 | 20 |

The p value for the genotype GA and AA was found to be Non-significant.

## IV. CONCLUSION

Since the CYP1B1 polymorphisms are inherited, they will dictate exposure levels to these E2 metabolites for life, which contribute to inter individual differences in ovarian cancer risk associated with estrogen-mediated carcinogenesis. The result of V432L polymorphism in CYP1B1 gene showed higher significance in cases than controls. As the current study involves a limited number of cases, further follow- up analysis involving a larger cohort is necessary to evaluate the true associations of the CYP1B1 and SULT1A1 polymorphisms in ovarian cancer.

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