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ABSTRACTS

Pharmacogenomics and toxicogenomics

349: Evaluating the effect of genotype and haplotype frequencies of ABCB1 (MDR1) polymorphisms in medically refractory epilepsy patients of an Indian population

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Epilepsy is a common disorder affecting approximately one percent of the population worldwide and has an estimated prevalence of 5.59/1,000 in Indian population. As many as 30% of the epilepsy patients do not have seizure control, in spite of adequate and appropriate pharmacotherapy (medically refractory epilepsy) which is a significant challenge for both patients and neurologists. The transporter hypothesis for intractility contends that the expression or function of multidrug transporters in the brain is augmented, leading to impaired access of anti epileptic drugs (AEDs) to CNS epileptogenic targets. A reported association of the single nucleotide polymorphism (SNP) C3435T of the ATP-binding cassette subfamily B member 1 (ABCB1, MDR1) gene, with refractory epilepsy is controversial due to inconsistencies in the attempted replications of the study. Here we present the results of an analysis of the effects of genotypes and haplotypes of three ABCB1 (MDR1) gene SNPs in a population of south western India. The study involved epilepsy patients with medically refractory and drug responsive epilepsy subgroups recruited from among the consecutive patients at the tertiary referral centre, Kasturba Hospital, Manipal, as well as normal controls. Drug resistance was defined as a terminal remission of less than 6 months with trials of two or more antiepileptic drugs at maximal tolerated doses. Drug responsiveness was defined as complete freedom from seizures for the past 6 months in epileptic patients treated with appropriate antiepileptic drugs. Three major coding region SNPs of ABCB1 gene, C3435T, G2677A/T, and C1236T were analyzed from the genomic DNA obtained from a total of 498 participants; 113 patients with medically refractory epilepsy, 129 having drug responsive epilepsy and 256 healthy controls. Method of analysis employed PCR-RFLP and confirmatory DNA sequencing. Statistically analyzed results showed no significant difference between the epileptic and control groups on the one hand and among epileptics, between drug refractory and drug responsive groups on the other; for allele and genotype frequencies of the three polymorphisms or any of their haplotypes. The results confirm lack of association of the three polymorphisms of ABCB1 with medically refractory epilepsy reported on at least three previous instances.

350: Mechanism of black tea polyphenols theaflavins and thearubigins induced apoptosis in human skin cancer cells: involvement of oxidative stress induced MAP kinase pathways

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Tea is one of the most common beverages consumed worldwide, and its possible beneficial health effects have received a great deal of attention. Various findings suggest that green tea and its polyphenolic components prevent a number of diseases including cancer. Interestingly although black tea is far more widely consumed than green tea, most of the studies on inhibition of carcinogenesis have been conducted with green tea and its components. Theaflavins (TF) and thearubigins (TR) are the major polyphenols of black tea. However, information is very limited on the anticancer activity of TF and TR. Our previous studies on human malignant melanoma cells, A375 showed that TF and TR potently induce apoptotic cell death via mitochondria mediated pathway and cell cycle arrest but not in the normal cells. These two polyphenols also increase the Bax/Bcl2 ratio, thus inducing apoptosis in our experimental model. It has been reported that green tea polyphenols can induce apoptosis by increasing the level of intracellular reactive oxygen species (ROS) in many cancer cells. In our present study we wanted to investigate whether TF and TR can induce ROS in skin cancer cells (A375) and whether they can modulate the MAP kinase pathway as well. We were also interested to know the relationship between MAP kinase pathway and apoptosis induction in our in vitro system. It has been

observed that TF and TR can induce production of intracellular ROS in A375 cells. Incubation with ROS scavenger can reduce the rate of apoptosis induction by TF and TR thus suggesting that ROS has got a vital role in TF and TR induced apoptosis in this cell line. The treatment with TF and TR on A375 cells does not show any change in the expression level of non-phospho form of three most important molecules of MAP kinase pathway viz. ERK1/2, JNK1/2 and p38. But the expression of phospho form of JNK1/2 and p38 have been increased in the first few hours upon the treatment of TF and TR and then decreased which signify that TF and TR may induce activation of stress activated MAP kinase pathways. Taken together our results strongly suggest that TF and TR executed apoptotic cell death in A375 cells via JNK and p38 MAP kinase pathways which are triggered by NAC-sensitive intracellular oxidative stress.

351: Genotype-phenotype correlation in β-Thalassemia patients (both major and intermedia) responding to hydroxyurea treatment and common β-Thalassemia mutations in the eastern Indian population

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β-Thalassemia, is the most common recessive inherited disorder in the Eastern Indian population, frequency is between 7 and 12%. It Is resulting from decreased erythroid beta-globin mRNA expression and imbalanced alpha/beta-globin chain synthesis which are manifest clinically by ineffective erythropoiesis and excessive haemolysis. Increasing levels of hemoglobin F (HbF) by pharmacological agents has like Hydroxyurea (HU), has been proposed to ameliorate the severity by improving in globin chain synthesis and to enhance HbF synthesis in patients with sickle cell anaemia and beta thalassemia. Among the many known mutations in Eastern India, five are common [like IVS1-5 G > C, Cod 8/9, Fr 41–42, Cod 15, β26]. The XmnI polymorphism was divided into two categories, (XmnI [+] and XmnI [-]) and the five common mutations into two groups (like, group I with IVS1-5 G > C and group II without IVS1-5 G > C). The -158 (C > T) polymorphism of the Gγ-globin gene (XmnI polymorphism) is known to ameliorate the severity of the disease because of its strong association with an increased production of HbF. The aim of our study is to analyze the influence of common β-Thalassemia mutations in patients with HU therapy. About 250 patients with β-thalassemia (both major and intermedia) were enrolled with informed consent to assess response to HU therapy. Diagnosis was based on quantification of total Hb by HPLC. Patients maintained Hb levels above 8 mg/dl and did not require blood transfusion were classified as major responders. Minor responders having Hb levels below 8 mg/dl required regular or occasional/spaced blood transfusion. Our study revealed statistically significant association of XmnI polymorphism with response to HU ($P = 0.001$). However, HU inducing HbF expression was inconsistent (more than 50% β0 are non-responders but with high HbF values and again some β+ without high HbF are good responders), suggesting the role of additional polymorphisms in other genes such as those involved in regulation of HbF expression, HU metabolism pathway and erythroid progenitor proliferation. Again, we can say that in addition to its known effects in stimulating gamma-globin production, HU may have a more general role in augmenting globin synthesis, including beta-globin in some thalassaemia intermedia patients who maintain the capacity to express normal beta-globin chains.

352: Genotypic variability of CYP2C9 and CYP2C19 in Indian population: correlation with pharmacokinetic based phenotype

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Drug-metabolizing enzymes (DMEs), drug transport proteins, and serum binding proteins are some of the factors which influence the disposition of drugs. DMEs are the most widely studied enzymes with respect to genetic variability related to drug response. Cytochrome P450 (CYP) enzymes are a large family of DMEs, and among others CYP2C9 and CYP2C19 exhibit clinically relevant genetic polymorphism. They are involved in the oxidative metabolism of most of the widely prescribed drugs, such as nonsteroidal anti-inflammatory drugs, proton pump inhibitors, etc. The genetic variation at CYP2C9 and CYP2C19 has a considerable phenotypic effect; the enzyme activity ranges from complete deficiency to extensive metabolism. The variation poses a challenge for safety and efficacy of drugs in human populations, especially in cases in which the therapeutic window is narrow, toxicity window is large and overlapping with the therapeutic window, time of treatment is critical, or the cost of treatment is high. To evaluate phenotype-genotype correlation for CYP2C9 and CYP2C19 in Indian population, we carried out relative bioavailability studies for the drugs metabolized by these CYPs to generate a pharmacokinetic (PK) based phenotype data and the samples were genotyped for the respective coding genes. Drugs included in the study were celecoxib, valdecoxib and diclofenac, which are substrates for CYP2C9, and lansoprazole, pantoprazole and omeprazole, which are substrates for CYP2C19. Around 10-fold difference in the Cmax and 34-fold difference in the AUC was observed among individuals when the PK based phenotypes were evaluated. Significant phenotype-genotype correlation was observed with drugs metabolized by CYP2C9 ($P \leq 0.05$) and CYP2C19 ($P \leq 0.001$). Genotyping and phenotyping tests to predict dose requirement are now increasingly being introduced during preclinical and clinical studies of new drugs. Thus phenotype-genotype correlation, as suggested in the present study, might be useful for better treatment regime.

353: Arsenic-induced gene expression changes in human peripheral lymphocytes and its association with carcinogenesis

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Arsenic is a widely known human carcinogen. Exposure to arsenic is alarmingly high in many countries, most notably India and Bangladesh, where more than 25 million people are chronically exposed to extreme arsenic levels. However, the molecular basis of arsenic-induced toxicity and its progression to cancer is poorly understood and still elusive. Since the manifestation of all cellular responses to arsenic is dependent on changes in expression, we set out to establish its effects on gene expression, through a microarray-based study in a human population currently exposed to alarming levels of arsenic. The blood lymphocyte gene expression profiles of individuals chronically exposed to arsenic with no manifest skin lesions were compared with subjects with arsenical skin lesions. This study is the first, to our knowledge, that examines the effect of arsenic on

genome-wide expression in humans; it also shows that blood arsenic is significantly associated with changes in transcription levels of several cell proliferation/cell-cycle-regulatory genes that have been implicated in carcinogenesis. We observed a subset of 471 differentially expressed genes in the skin-lesion manifestation group; significantly, they included some stress response proteins like, Hsp40, HspC035, HspC038 and HspC039 that can be putative potential biomarkers of arsenic exposure. In addition, there was a boost in expression of key cell cycle regulatory genes like cellular Cyclin A, Cyclin C, Cdc2, Cdk4, Cdk5, Cdk6 and Cdk7. These genes play an active part in cell cycle progression, and evidence of increased expression of cellular cyclins and Cdk's upon arsenic insult can be indicative of enhanced or uncontrolled cell division. It thus underlines the carcinogenic potential of arsenic. The results from microarray experiment were further validated by qRT-PCR and in vitro experiments. Although transcription changes in all identified genes have not previously been linked to arsenic carcinogenesis, their association with carcinogenesis in other systems suggests that these genes may play a role in any stage of arsenic-induced carcinogenesis and can be considered potential biomarkers.

354: Arsenic induced premature senescence: a biomarker study in exposed population from West Bengal, India

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Arsenic is an environmental contaminant that causes cancer, developmental retardation and other degenerative diseases and thus is a serious health concern worldwide. In India, West Bengal, 9 out of 18 districts are contaminated with ground water arsenic at a concentration much above the threshold limit recommended by WHO (10 μg/l). Although, arsenic is a well-known carcinogen, limited knowledge exists about its potential to induce stress-induced premature senescence. Normally, somatic cells enter a state of irreversibly arrested growth after a finite number of divisions, termed as replicative senescence, characterized by gradual shortening of telomeres. On the contrary, premature senescence is caused by external or internal stresses, resulting into altered physiological, biochemical and structural changes such as increased beta-galactosidase activity (histochemically detectable at pH 6), rapid shortening of telomeres and altered telomere behaviour. In this study, we wanted to find out whether arsenic can induce these characteristic changes of premature senescence in peripheral blood lymphocytes. As exposed area, we selected Murshidabad district with heavy contamination of groundwater arsenic; as arsenic-unexposed area, we selected Midnapore district where arsenic contamination has not yet been reported. As study subjects, we recruited 21 arsenic exposed individuals (cases) and 21 age-sex matched control individuals who were completely unexposed to arsenic. We measured the expression of senescence-associated beta-galactosidase (SA-beta-Gal) in peripheral blood lymphocytes. We also measured telomere length by Telomere Restriction Fragment (TRF) analysis through genomic DNA isolation, digestion with restriction enzyme, electrophoresis, Southern transfer and detection by chemiluminescence method. We also analysed chromosomal aberrations to find out specific aberrations at telomere region. In our results, SA- beta gal positive cells were found to be significantly higher ($P \leq 0.05$) in exposed individuals than control individuals. Mean TRF length were found to be significantly lower ($P \leq 0.001$) in cases than controls. Cytogenetic analysis revealed that arsenic exposed individuals exhibit several aberrations at telomeric region. Thus, this study indicates that arsenic induces premature senescence that is reflected by drastic telomere shortening and abnormal changes in the telomeric regions of the chromosomes.

355: Genetic variations in TCF7L2 influence therapeutic response to sulfonylureas in Indian diabetics

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Type 2 diabetes prevalence is increasing worldwide, expected to reach 333 million diabetics by the year 2025. Sulfonylureas are widely used to treat type 2 diabetes and there is a considerable inter-individual variation in the hypoglycaemic response to sulfonylureas. Genetic variants in the gene encoding for Transcription factor-7-like 2 (TCF7L2) have been associated with type 2 diabetes and impaired β cell function. TCF7L2, also known as Transcription factor-4 (TCF-4), is a nuclear receptor for CTNNB1 (previously known as β-catenin), mediating the canonical Wingless-type MMTV integration site family (WNT) signalling pathway. There are several known mechanisms for the involvement of WNT signalling in both insulin secretion and action, as well as in cell differentiation and maturation. It has been suggested that the WNT/TCF7L2 pathway in enteroendocrine cells may regulate intestinal proglucagon gene expression. Thus TCF7L2 variants may modify type 2 diabetes susceptibility and hypoglycaemic response to sulfonylureas through alterations in glucagon-like peptide-1 (GLP-1) that is linked to physiological insulin secretion. To date, pharmacogenetic studies on the therapeutic response within diabetes have been limited. This study aimed to study the effect of variations in TCF7L2 on therapeutic response to sulfonylureas. The effect of TCF7L2 rs12255372, rs7903146 and rs4506565 genotypes on glycaemic response was observed in 125 diabetic patients treated with sulfonylureas and sulfonylureas along with metformin. Postprandial blood sugar concentrations were used as phenotypic marker. Across the whole cohort 60% of sulfonylurea users did not achieve a target postprandial blood sugar concentrations of <160 mg/dl. Genotype influenced response to sulfonylureas, with more treatment failure in the TT homozygotes in case of rs12255372 and rs4506565. We have seen that GG genotype at rs12255372 favorably influences initial treatment success with sulfonylurea therapy in patients with type 2 diabetes ($P \leq 0.05$). At rs12255372, 70.5% GT or TT genotype failed to achieve therapeutic target, an absolute difference of 19% compared to GG homozygotes. Our preliminary data show that influence of rs4506565 and rs7903146 on therapy was not statistically significant, however, genetic variation at rs12255372 has a direct correlation with therapeutic success with sulfonylureas in type 2 diabetes.

356: Gene expression profiling in lymphocytes of arsenic exposed individuals

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In West Bengal, about six million people are exposed to arsenic through drinking water. Human exposure to this toxic metalloid is

long known to result in hazardous health outcomes, including cancers of skin and other internal organs. Although skin lesions are recognized as the most sensitive end points of chronic arsenicism, only 15–20% of the exposed individuals show arsenic-induced skin lesion, suggesting that genetic variation might play an important role in arsenic susceptibility, toxicity and carcinogenicity. Hence, it is of prime interest to identify the susceptible factors through gene expression, that render one group susceptible to arsenic toxicity, while others remain skin-asymptomatic even after exposure at a similar extent. Therefore, in the present study, we have studied the gene-expression profile in arsenic exposed (skin lesion and no skin lesion) and unexposed individuals. For this study, age and sex matched exposed individuals with or without arsenic-induced skin lesions and unexposed individuals were recruited. RNA was extracted from the whole blood and gene expression analysis was performed using Agilent Whole Genome DNA Microarrays. From these studies, we have identified a unique repertoire of genes that are overexpressed in arsenic exposed individuals with skin lesions but not in other groups. In addition, we have identified genes that are overexpressed in both arsenic exposed groups as compared to controls. Our data shows that it is possible to identify genes as putative candidate molecules that may play a role in susceptibility and/or pathogenesis of arsenic-induced skin lesions.

357: An acute antidepressant pharmacogenomic study and association of *ADM*, a paroxetine-regulated gene, with antidepressant response

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Aim: Genes which are regulated by antidepressants may associate with antidepressant response or be susceptibility factors for mood disorders. We decided to explore acute antidepressant-induced gene expression changes in a serotonergic cell line to find candidate genes for association studies.
Methods: Differentiated RN46A cells were exposed to the antidepressant paroxetine for 36 h. RNA was taken for microarray analysis using Affymetrix rat 230 2.0 GeneChips and quantitative PCR (Q-PCR) assays were developed to validate transcriptional changes. From the gene expression study, the human homologue of *Adm* was screened for genetic variation and a single nucleotide polymorphism (SNP) was identified. Reporter gene assays examined expression of the two alleles and association studies were carried out in a family study of depression.
Results: 253 genes were found to be differentially expressed after paroxetine treatment. The expression of two genes (*Id2* and *Ucn2*) were validated by Q-PCR methods and the transcriptional changes of a further three genes (*Adm*, *Bnip3* and *Ankrd37*) approached conventional statistical significance ($P < 0.1$). *ADM* was chosen as a candidate gene for association studies because of its relatively large paroxetine-induced expression change and the previous association of ADM levels with bipolar and other psychiatric disorders. Screening of the upstream and 5′ region of *ADM* identified -1923 C > A SNP (rs11042725). The C allele of this SNP had less expression than the A allele in reporter gene assays in RN46A cells and the C/C genotype was associated with less likelihood of response to paroxetine in depressed individuals.
Conclusions: This study needs to be replicated in an independent cohort before the association of *ADM* -1923 C/C with response to paroxetine can be confirmed. However, this work has shown that the pharmacogenomic approach can be successfully used to identify genes which may associate with antidepressant response.

358: Cytotoxic and apoptogenic effects of coumarin derivatives on human lung adenocarcinoma cell line

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Natural dietary agents including fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancer. Coumarins, naturally occurring benzopyrene derivatives have been shown to possess diverse pharmacological activity. In the present study we made an attempt to investigate the antiproliferative potential of coumarin derivatives on human lung adenocarcinoma cell line using biochemical and proteomic approaches. We selected 7,8 diacetoxy-4-methyl coumarin (DAMC), 7,8 dihydroxy-4-methyl coumarin (DHMC), and 7,8 diacetoxy-4-methyl thiocoumarin (DAMTC) for our study. Our results demonstrated that DAMC, DHMC and DAMTC inhibited the growth of A549 cells and induces apoptosis as was evident from annexin assay, flow cytometric analysis and TUNEL assay. More interestingly these coumarin derivatives had very little effect on normal PBMC cells. Induction of apoptosis occurred through mitochondrial pathway in a ROS independent manner. We observed that these coumarin derivatives induce apoptosis in A549 cell line by affecting multiple cellular pathways such as MAP kinase, NF-kappa B and akt pathway. Our proteomics result using 2D electrophoresis further proves the proapoptotic nature of DAMC, DHMC and DAMTC in A549 cell line. Our findings may offer new insights into the antiproliferative activity of DAMC, DAMTC and DHMC in A549 cells which will be important to fully exploit the potential of these drugs as chemotherapeutic agents.

359: Pharmacogenomics of first line anti-epileptic drugs (AEDs) in Indian population

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Responses among patients to anti-epileptic drugs (AEDs) are highly variable, with respect to both drug efficacy and safety. Earlier studies have focused on genes whose products may play a putative role in AED pharmacokinetics and pharmacodynamics particularly drug metabolizing enzymes, drug transporters and ion channel subunits. Phenytoin and carbamazepine are anticonvulsants, exhibiting nonlinear pharmacokinetics with large interindividual differences. The interindividual differences in dose response may partly be explained by known genetic polymorphisms in the metabolic enzymes belonging to cytochrome P-450 family, hydrolases and transferases but a large deal of individual variability still remains unexplained. Part of this variability might be accounted by variable uptake of phenytoin and carbamazepine by ATP-binding cassette transporters (ABCB1, ABCC1 and ABCC2) and

sensitivity of drug targets (SCN1A, SCN1B, GABA-A). The aim of the study is to determine the allele frequencies of genetic variants of these genes in the Indian epileptic patients that might influence pharmacokinetic and pharmacodynamic response to phenytoin and carbamazepine. We undertook the single-nucleotide polymorphism (SNP) screening of 19 genes (230 SNPs selected from NCBI) and genotyped an average of $\sim$12 SNPs per gene, with an average density of 1 SNP per 4 kb. Among these 230 SNPs, 89 were non polymorphic and 141 SNPs (102 intronic, 13 non-synonymous, 14 synonymous, five untranslated region, five 5′ flanking region and two 3′ flanking region) were found to be polymorphic in our studied population. The allelic frequencies for the polymorphic SNPs were evaluated in the 295 unrelated epileptic patients and were found to be in conformance with Hardy-Weinberg equilibrium. Understanding of function of these genes and AED response will depend upon clinical investigations together with associated genetic variation information. This information on specific AED pharmacokinetics and pharmacodynamics might lead us to the basis of clinical assays to predict the most likely response to a drug in an individual patient.

360: Interindividual variability in drug response to risperidone treatment in schizophrenia: role of dopaminergic pathway gene polymorphisms

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Alterations in the dopamine transmission and receptor density have been hypothesized in the pathophysiology of schizophrenia and therapeutic response to antipsychotic medication. Genes involved in regulation of dopamine bioavailability may thus be important, and have been evaluated as candidate genes. We investigated a panel of polymorphisms from the DRD2, COMT and BDNF genes, involved in the dopaminergic pathways, in 117 risperidone-treated south Indian schizophrenia patients. Treatment response was assessed using Clinical Global Impressions (CGI). Patients who showed improvement after treatment with risperidone with a CGI score of 2 or less were classified as good responders. Patients who continued to have a score of 3 and above were classified as poor responders. We carried out single-locus and haplotype-based association analyses of DRD2, COMT and BDNF gene with drug response. We also examined the gene-gene interactions to identify combinations of polymorphisms that best predict the drug response by performing logistic regression analysis. Our results demonstrated a significant association of SNPs for COMT (rs4633-genotype: $P = 0.047$ and allele; $P = 0.038$; OR = 1.79, 95% CI = 1.02–3.14; rs4680-genotype: $P = 0.047$) before Bonferroni correction. Haplotype analysis with three markers from COMT gene (rs737865, rs4680, rs165599) showed a significant association of one haplotype with good responders (TAA, $P = 0.002$), and one with poor responders (TGG: $P = 0.008$). Logistic regression analysis revealed one of the models with combination of two polymorphisms (rs1801028, DRD2 and rs6265, BDNF) which showed strongest association with response to risperidone ($P = 0.009$). The present study shows that the dopamine pathway gene polymorphisms may influence response to risperidone in schizophrenia patients. However, the study needs to be replicated in a larger sample set for confirmation, followed by functional studies.

361: Role of NBN gene polymorphisms in DNA repair

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Accurate maintenance of nuclear DNA is a critical function of every cell. Cells remove several kinds of spontaneous and environment-induced DNA damages by employing diverse DNA repair mechanisms and maintain the integrity of genome. Interplay of different DNA repair pathways provides the most robust defense for the cell. As there is more number of repair enzymes, the variation in their expression, the existence of multiple alleles at loci of those enzymes may result in differential susceptibility of individuals. Polymorphism in DNA repair genes may be associated with differences in the DNA repair efficiency of DNA damage and influence individuals risk of cancer because of variant genotype might destroy or alter the repair function. Reduced repair capacity caused by mutations/polymorphisms in DNA repair genes is linked to cancer predisposition, developmental abnormalities, neurological disorders, and premature aging syndromes. In this direction, Nijmegen breakage syndrome 1 gene, nibrin (NBN) is employed in the present preliminary study to understand its influence and the role of its polymorphisms in DNA repair in human peripheral blood mononuclear cells. DNA was isolated from 150 control samples. The genotyping was performed at two positions such as NBN-8360 and NBN-30537 using PCR-RFLP method. The results have shown that the genotype frequencies of G/G, G/C and C/C are 31, 58 and 11%, respectively for NBN-8360. On the other hand, the frequency of G/G is 97% and G/C is 3% for NBN-30537. However, the C/C genotype was not observed in the screened individuals. DNA repair capacity was studied by Fluorometric analysis of DNA unwinding technique (FADU) and RNA expression was studied by using RT-PCR. The results of these studies will be discussed.

362: Pharmacogenetics on drugs for rheumatic diseases

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Pharmacogenetic studies on effectiveness and toxicity of various drugs have been performed widely for the personalized medication based on individual genetic information. Over the past few years, we have studied the genetic backgrounds for diverse responses of three different drugs in treatments of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and osteoarthritis (OA). Firstly, the clinical response to etanercept therapy in RA patients was significantly associated with the polymorphism at position-857 of the tumor necrosis factor α gene. Patients with the minor allele in the SNP responded better to etanercept therapy than those with the major-allele homozygotes, indicating that this SNP could be a useful genetic marker for predicting responses. Secondly, emetic adverse events of tramadol-acetaminophen combination therapy for OA patients primarily depended on polymorphisms in a drug-metabolizing enzyme and a drug target. Patients having slow-metabolizing alleles and/or low-expression allele of the opioid receptor gene less likely suffer from nausea or vomiting. Finally, we are investigating the genetic variants that are associated with the efficacy and toxicity of cyclophosphamide therapy in SLE patients. The drug response or adverse events was not significantly associated with polymorphisms in glutathione S-transferase.

363: Role of GSTM1, GSTP1 and GSTT1 polymorphisms in the succeptibility to acute lymphoblastic leukemia and the influence of SNPs in the TPMT gene to the tolerance of thiopurines in the ALL patients

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Background: Acute lymphoblastic leukemia is the most common pediatric malignancy. The origin of this disease can be explained by a combination of genetic and environmental factors. Glutathione-s-transferases are a multi-gene family of enzymes involved in the detoxification of wide variety of environmental carcinogens. Thiopurine S-methyltransferase (TPMT) catalyses the S-methylation of thiopurine drugs. Single Nucleotide Polymorphisms in the TPMT gene have been identified which correlate with a low activity phenotype. Our aim is to assess the role of GSTM1(present/null), GSTP1(Ile105Val) and GSTT1(present/null) polymorphisms in the succeptibility to acute lymphoblastic leukemia and to assess the influence of TPMT single nucleotide polymorphisms to the tolerance of thiopurines in the ALL patients.
Materials and methods: Our study includes 87 immunophenotyped cases (below 25 years of age) and 150 cord blood controls. Polymorphisms were genotyped by PCR (GSTM1, TPMT(G238C)), PCR-RFLP for TPMT(G460A) and RQ-PCR Allelic discrimination assay for GSTP1.
Results: We found a significant increased risk of ALL with GSTM1 null genotype. (OR = 2.11, 95% CI = 1.14–3.89). No significant risk is found with GSTP1 ($P = 0.477$). TPMT(G238C) and TPMT(G460A) are rare polymorphisms and were not detected in our patients. TPMT(A719T) and GSTT1 genotyping are being done and will be discussed.

364: Pharmacogenomics and predictive therapy for complex diseases

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Therapeutic efficacies and side effect profiles of drugs differ among individuals. Genetic variations in genes encoding components of drug metabolizing enzymes, transporters, primary and secondary targets of metabolites, and downstream pathways are all considered to underlie this difference. Pharmacogenomics of drugs prescribed for common diseases such as epilepsy, mental disorders, etc. is needed for individual specific therapy (personalized medicine) as these diseases have emerged as a major public health problem in recent years. However, personalized medicine is likely to be very expensive and unaffordable in developing world. The pharmacogenomics approach taken here is to make drugs affordable by keeping old and cheap drugs and reviving drugs withdrawn from the market because of their side effects. We have selected 24 Indian populations representing major four groups (Journal of Genetics, Vol. 87, No. 1, April 2008) and genotyped 552 individuals at 488 SNP locus covering 112 genes. These include 28 genes coding for DMEs of both phase I (oxidation or reduction) and Phase II (conjugation) drug metabolism, nine drug transporter genes and 75 drug targets. The detailed analysis of these results will be presented.

365: Pharmacogenomic study of drug transporter ABCB1 and drug target SCN1A in epilepsy patients from North India

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Introduction: Epilepsy is a common, serious, and treatable neurological disorder, yet current treatment is limited by lack of complete seizure control in a significant proportion of patients. Some recent genetic studies have shown that ion channels, drug transporters and receptors play major role in drug response as well as causation of epilepsies. Studies of genetic variations in genes encoding drug transporters and antiepileptic drugs (AEDs) targets may alter the clinical response to these AEDs and thus account for some of the interindividual variations in AED response.
Objective: Aim of the current study was to evaluate influence of ABCB1 3435C > T (rs1045642), 1236C > T (rs1128503) and SCN1A 3184A > G (rs2298771) polymorphisms in clinical response to epilepsy therapy.
Materials and methods: A total of 315 epilepsy patients, including 93 non responders and 222 responders were genotyped using standard PCR-RFLP methods. The association was assessed by using logistic regression, odds ratio and 95% confidence interval were calculated and $P < 0.05$ was considered significant. AED levels were measured in 25% of patients using HPLC, to confirm drug compliance.
Results: Mean age of drug resistant epilepsy patients were 23.49 ± 11.88 and 23.89 ± 10.83 for responsive patients. Age of appearance of first seizure was 15.96 ± 9.624 in drug responsive groups and 13.67 ± 10.59 in drug resistant patients. SCN1A AG genotype [OR = 1.137 95% CI (0.693–1.867) $P = 0.611$] and GG genotype [OR = 1.137, 95% CI (0.693–1.867) $P = 0.895$] frequencies did not differ significantly between drug refractory epileptic patients in comparison with drug responsive patients. Furthermore, we did not observe any association of ABCB1 1236C > T polymorphism for CT genotype [OR = 0.99, 95% CI (0.468–2.102) $P = 0.98$] and for CC genotype [OR = 0.946, 95% CI (0.426–2.102) $P = 0.891$].The patients with ABCB1 3435C > T polymorphism, CT (OR = 1.926, 95% CI = 0.860–4.310)] and TT (OR = 1.528, 95% CI = 0.662–3.524) showed higher risk for drug non-responsiveness but the differences were not statistically significant ($P > 0.05$) when compared with drug responsive patients.
Conclusion: Our study suggests that ABCB1 3435C > T (rs1045642), 1236C > T (rs1128503)) and SCN1A 3184A > G (rs2298771) polymorphisms do not significantly influence drug responsiveness in north Indian epilepsy patients.
Acknowledgement: Grant support received from DBT and DST (India).

366: Investigating the role of polymorphisms in ABCB1 and MTHFR with teratogenicity of antiepileptic drugs

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Teratogens terminate the pregnancy or produce congenital malformations. Most drugs taken by pregnant women can cross the placenta and expose the developing fetus to their pharmacologic and teratogenic effects. Congenital abnormalities and impaired development in childhood are attributable to fetal exposure to antiepileptic drugs (AEDs). Women taking AEDs carry a two to sevenfold higher risk of congenital malformations than do the general population. Fetal exposure to AEDs may be influenced by drug transporting proteins in the placenta. ABCB1 is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity and is expressed in a wide variety of tissues including placenta. The SNPs in ABCB1 gene may have an effect on the drug efflux properties of the MDR protein. AEDs can impair folate absorption. MTHFR plays a key role in cellular folate metabolism. It catalyzes the reduction of 5,10-methyltetrahydrofolate and allows the re-methylation of homocysteine to methionine. Folic acid affects the neural tube closure and has considerable role in circumventing Neural Tube Defects (NTD). We have followed case–control study design and had two groups of controls (healthy control and patient control). The case group comprised of women with epilepsy who had offspring with various malformations. The healthy controls were individuals with no background of epilepsy and patient control comprised of epileptic women with healthy offspring. The AEDs prescribed to the patient group in general were Phenobarbitone, Valproic acid, Carbamazepine, Clobazam, Ethosuximide, Primidone and others. Seven SNPs in ABCB1 gene (T129C, G-1A, C139T, C1236T, T-76A, G2677T and C3435T) were analysed. Two SNPs in MTHFR- C677T and A1298C which are implicated in decreased enzyme activity were also analysed. Various statistical tools were used to compare the Hardy-Weinberg's equilibrium, allele frequency, genotype frequency, haplotype frequencies and linkage disequilibrium (LD). Allelic, genotypic haplotypic and LD patterns were evaluated between the cases-controls, monotherapy-polytherapy and siezure-nonsiezure groups. In our study, allelic and genotypic observations do not indicate any significant association in any of the groups. However, a haplotypic association in MTHFR gene was observed with malformations. Similar significant haplotypic association was observed with seizure in ABCB1gene. Differences in the LD patterns were also observed between epileptic controls and malformation group in ABCB1 gene.

367: Pharmacogenomic study of drug transporter ABCB1 and CYP3A4*1B, CYP3A5*3 polymorphisms associated with daily dose requirement of cyclosporine A to prevent renal allograft rejection in North India

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Introduction: Cyclosporine A (CsA) is a substrate of cytochrome P-450 3A (CYP3A) and ATP binding cassette subfamily B member 1 (ABCB1). Inter-individual heterogeneity in the expression of CYP3A4, CYP3A5 and ABCB1 genes has been suspected to be one of the factors resulting in CsA pharmacokinetic variation.
Objective: The present study explored the association of CYP3A4, CYP3A5 and ABCB1 SNPs on CsA dose requirements and dose-adjusted C2 levels (CsA level/daily dose requirement) in renal allograft recipients. Methods: Daily doses (mg/Kg/day) and dose-adjusted C2 levels (μg/ml per mg/Kg/day) at 1 and 3 months post transplantation for 155 recipients on CsA immunosuppression based therapy were compared with frequency distribution of CYP3A4*1B-290A > G, CYP3A5*3 c.6986A > G, and ABCB1 c.1236C > T, c.2677G > T, c.3435C > T. C2 levels (μg/ml) in whole blood was measured by EMIT assay.
Result: The *1/*1 genotype of CYP3A4*1B exhibited lower dose-adjusted C2 levels as compared to *1/*1B or *1B/*1B genotypes at 1 and 3 months post transplantation (Mann–Whitney U test; $P = 0.025$ and $P = 0.009$). The dose-adjusted C2 levels were also lower in ABCB1 c.2677G > T GG genotype (1 month, $P = 0.009$; 3 months, $P = 0.043$). The GG genotype was further associated with lower allograft survival as indicated by Kaplan-Meier analysis ($P = 0.021$). The CYP3A5*3 polymorphism was not linked with CsA dose requirement in the present study cohort.
Conclusion: The identification of patients with *1/*1 genotype of CYP3A4*1B and GG genotype of ABCB1 c.2677G > T may have a significant impact on allograft outcome clinically and may be helpful in providing pre-transplant pharmacogenetic information to individualize cyclosporine A dosing to prevent allograft rejection.
Acknowledgement: Grant support received from DBT and CSIR (India).

368: Association between cholinesterase activity and human paraoxonase (PON1) polymorphisms in workers exposed to organophosphate

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Serum paraoxonase (PON1) hydrolyses toxic oxons of a variety of organophosphates (OPs). PON1 displays large inter-individual activity largely owing to genetic polymorphisms. In this study, we studied the relationship between two PON1 polymorphisms on both cholinesterase and PON1 activities in workers exposed to OP-containing pesticides. PON1 polymorphisms Q192R and L55M were determined by PCR-RFLP method. PON1 activity towards two substrates (paraoxon and diazoxon) was analysed concurrently with cholinesterase activity among these workers. PON1 activity exhibited substrate dependent polymorphism. AChE levels were similar between exposed and non-exposed workers. PON1 activity seemed to be higher among exposed workers. Multiple linear regression for AChE activity yielded BMI as a significant factor. Controlling for BMI, the results indicate that an increase of 1.0 μg/dl in PON1 activity was associated with an increase of 6.17 U/l in serum PChE levels. PON1 activity varied significantly over the three different race groups in our sample, namely Chinese, Malays and Indians who make up the major races in Singapore. Chinese and Malays had higher frequencies of the PON1 allele more effective against paraoxon. We postulate that upregulated PON1 activities could have contributed to the fact that AChE was not lowered in exposed workers. We can also conclude that pesticide workers in Singapore are practicing proper personal protective equipment along with other guidelines to ensure non-hazardous exposure to OPs.

369: HLA genotypes in carbamazepine-induced severe cutaneous adverse drug response: difference between Japanese and Han-Chinese

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The antiepileptic drug, carbamazepine has a high incidence of drug eruption. Recently, the HLA-B*1502 has been shown to tightly associate with the rare and most severest form of adverse cutaneous reaction (cADR), namely Stevens–Johnson's sydrome (SJS) and Toxic Epidermal Necrolysis (TEN). However, following studies from Europe have shown that this biomarker is effective only in Asian ancestry suggesting that ethnicity may matter. We studied HLA genotypes in twenty-two Japanese patients with carbamazepine-induced severe cADR, who needed hospitalization. Genotyping of HLA in all of the cases revealed that none of them possessed the B*1502 genotype including two cases of SJS. Other retrospective study of SJS patients have also failed to identify this biomarker in Japanese. To this end, a nationwide multi-center collection of SJS patients has been organized in Japan. The update of the project will be presented. It appears that B*1502 dependent carbamazepine-induced SJS is much rare in Japanese compared to those in Han Chinese.

370: Polymorphisms in statin metabolism pathway genes predict statin mediated LDL cholesterol lowering in coronary artery disease patients

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Statins can substantially lower plasma LDL cholesterol (LDLC) and reduce risk for coronary heart disease, but their efficacy varies among individuals. Prior studies have reported various genes, often singly for association with drug response, with contradictory results. Few studies have systematically tested for interactions between single nucleotide polymorphisms (SNPs) between genes. To test whether this variation is related to cumulative effects of gene-gene interactions, we examined the genotypic variations and haplotype associations in several candidate genes of statin metabolism pathway as the potential determinants of drug responsiveness in coronary artery disease (CAD). 265 newly diagnosed CAD patients, with an LDLC (mean 131 mg/dl) were treated with 20 mg/day Atorvastatin for 6 weeks. We genotyped 11 SNPs of four genes involved in statin, cholesterol, and lipoprotein metabolism CETP-C629A, TaqIB, I405 V, ApoAI-PstI, G75A, C2500T, ABCB1-G2677T/A, C3435T, A41G and CYP7A1-A-278C, A-204C by PCR-RFLP assays. Patients were grouped as responders (LDLC < 100 mg/dl) and non-responders (LDLC > 100 mg/dl) to atorvastatin according to the National Cholesterol Education Program and Adult Treatment Panel III guidelines. Genotypic and haplotypic interactions were studied using multifactor dimensionality reduction MDR method and PHASE 2.1, respectively. The frequency of genotypes ApoAI -2500CC,PstIP1P1, CETP TaqIB1B2, 629CC and ABCB1 3435CC were significantly higher in responders ($P < 0.05$), and frequency of Apo AI-PstI P1P2, CETP-TaqI B2B2, 405IV,629AA and ABCB13435TT genotypes were significantly higher in non-responders. Frequency of haplotypes CETP: B1VA, ApoA1-P1TA, P2CA, ABCB1:CGT were found to be significantly higher in responders ($P < 0.05$). The linkage disequilibrium was significantly low across the studied SNPs ranging from $D = 0.04$–0.20. MDR analysis showed three loci SNP combination (TaqIB1B2-629CC-3435CT) to be the best genotype combination model predicting LDLC lowering response (OR = 5.5078, 95%CI = 2.64–11.48). This study is the first to comprehensively investigate variation in multiple statin metabolism genes and LDL lowering by statins. SNPs in ABCB1, APOAI, and CETP predict response to Atorvastatin. This study highlights the use of a multigene strategy in pharmacogenetic studies.

371: Polymorphism of serine hydroxymethyltransferase gene (SHMT1 C1420T) and risk of colon cancer: an Indian case–control study

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Serine hydroxymethyltranferase (SHMT) gene encodes a vitamin B6-dependent enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate(THF) to glycine and methylene tetrahydrofolate. SHMT1 plays a pivotal role in providing one-carbon units for purine, thymidylate and methionine synthesis in addition to metabolic functions. Based on the role of SHMT in the provision of one-carbon units for multiple folate pathways, disturbances in enzyme activity due to SHMT1 C1420T polymorphism could mimic a folate deficiency by reducing the one-carbon moieties available for both remethlylation of homocystein and DNA synthesis, making this an ideal gene candidate to study. Polymorphisms in cytosolic serine hydroxymethyltranferase (SHMT1 C1420T) have been reported to modulate the risk of colon cancer. We examined the associations between susceptibility to colon cancer and this polymorphism. A hospital based prevalent case–control study was conducted. One hundred patients with histopathologically confirmed colon cancer and 100 control subjects without cancer were evaluated. This case–control study consisted of patients and population controls of both sex and age group above 18 years. The genotype analysis was performed using PCR-RFLP analysis and DNA sequencing from the genomic DNA obtained from patients and control population samples. An unconditional logistic regression model was applied for estimating the odds ratio (ORs) and 95% confidence interval (CIs) to find the relative risk. We also carried out multisequence alignment for exon 12 of human SHMTI gene using CLUSTAL-X to find the evolutionary relationship. Secondary structure prediction was also carried out using secondary structure prediction software GOR4. Prevalence of this polymorphism in control population was correlated with patient group. There was a difference in genotype frequency between patients and control groups which were statistically insignificant this may be due to small sample size. Even through CT and TT alleles contributed to relative risk of colon cancer it was not statistically significant. However, further studies have to be done by increasing the sample size to get a stronger association.

372: Genetic variations and haplotypes of the 5′ regulatory region of *CYP2C19* in South Indian population

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CYP2C19 is involved in the metabolism of various drugs like proton pump inhibitors, tricyclic antidepressants, proguanil and nelfinavir. It is expressed polymorphically with about 21 variant alleles. Genotype-phenotype association studies have shown marked deviations suggesting the presence of other variations in the intronic and 5′ regulatory region which includes the promoter. The polymorphisms in the 5′ regulatory region may affect the transcription of the enzyme and alters the enzyme levels. The aim of the study was to identify the genetic polymorphisms in 5′ regulatory region of *CYP2C19* among South Indian population. *CYP2C19* 5′ regulatory region was amplified and sequenced from the genomic DNA of healthy volunteers ($n = 58$) of South Indian origin using gene specific primers. In addition the two known non synonymous single nucleotide polymorphisms, 681G > A (splicing defect, *2 allele) and 636G > A (*3 allele) were also detected by PCR-RFLP method. Individual sequences were constructed into single contigs and were aligned with the reference sequence. Hardy-Weinberg equilibrium, linkage disequilibrium, and haplotype analysis was done to characterize the interaction between various polymorphisms. Genetic analysis revealed the existence of 14 variations including 8 novel ones in the 5′ regulatory region. Identified variations and their frequencies were: −98T > C (28.4), −779A > C (16.4), −806C > T (2.6), −828T > A (2.6), −833del > T(9.5), −889T > G (10.3), −934del > T (3.5), −1041A > G (0.0), −1051T > C (1.72), −1289T > G(3.4), −418C > T (1.7), −1442T > C (12.1), −1498T > G (25.0), −1558T > G (2.6). All the variations were found to be in Hardy-Weinberg equilibrium. The frequencies of *1(60.3), *2(37.1) and *3 (2.5) alleles in the study population were similar to the previous reports. Forty three haplotypes were constructed, and among them 13 haplotypes were found to be in higher frequencies (>1%). LD analysis showed strong linkage between several variations identified in the gene. Fourteen polymorphisms including 8 novel ones in *CYP2C19* 5′ flanking region were reported for the first time in an Indian population. Results from this study provide additional information for genotyping of *CYP2C19* in South Indian population and probably in the Indian population. Further functional characterization of the individual SNPs may explain the molecular mechanism, by which *CYP2C19* can be induced in clinical setting and the consequences of genetic variability in the promoter region of *CYP2C19*.

373: Apolipoprotein E polymorphisms influence the response of lipids to atorvastatin in hypercholesterolemic patients with coronary artery disease

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Polymorphism in apolipoprotein E gene (APOE) is the most relevant genetic factor for pharmacogenomic analysis of statin therapy. Apolipoprotein E (ApoE) is known to modulate lipid and lipoprotein homeostasis and the genetic heterogeneity in apoE has been associated with lipid profile and risk of coronary artery disease (CAD). However, the association of APOE genotypes with response to statins is still inconclusive. Therefore, we sought to examine the association of APOE genotypes with baseline lipid values, Atorvastatin-induced LDL-cholesterol response and incident CAD.200 clinically confirmed, untreated CAD patients were given atorvastatin (20 mg/day) treatment for 6 weeks. Serum lipid levels were measured before and after 6-weeks of atorvastatin therapy. APOE was genotyped by PCR-RFLP. The frequency of APOE 3/4 genotype and the APOE ε4 allele was significantly higher in the patients compared to controls with an odds ratio (OR) of 4.68 ($P < 0.001$) and (OR = 6.62, $P = 0.0009$) respectively. APOE2/4, APOE2/2 and APOE3/4 genotype carriers showed significantly greater reduction in Total Cholesterol. APOE3/4 showed decrease in Triglyceride levels. Subjects with APOE2/4 genotype had significantly greater LDL-C lowering ($P < 0.05$) as compared with carriers of other genotypes. Our study demonstrates that APOE genotypes influence inter individual variability of LDL-cholesterol lowering response to Atorvastatin.

374: ACE gene polymorphism: a potent risk factor for End stage renal disease among North Indians

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Background: End stage renal disease (ESRD) is a complex disorder encompassing a large variety of phenotypes. Each phenotype is a result of an underline kidney disease and superimposing environmental and genetic factors. The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and their interaction. In such a situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin converting enzyme-1 (ACE-1) is an important component of RAS which determines the vasoactive peptide Angiotensin-II. Our study was designed to see the role ACE gene polymorphism individually and synergistically in the progression of renal failure.

Material and methods: In the present study, we have investigated 258 ESRD patients and 569 normal healthy controls from north India. The genotype of ACE gene was determined by PCR and agarose gel electrophoresis.

Results: The difference of DD and II genotypes was found highly significant among the two groups ($P \leq 0.0001$, OR = 3.97, 95%CI = 3.17–4.97). The combined genotype DD versus ID + II comparison validated that DD genotype is a high risk genotype for ESRD ($P \leq 0.0001$, OR = 5.24, 95%CI = 3.47–7.92). However, no correlation was obtained for different biochemical parameters of lipid profile and renal function among DD and non DD genotype. Interestingly, approximately 87% of the DD ESRD patients were found hypertensive in comparison to the 65% patients of non DD genotype.

Conclusions: Based on these observations we conclude that ACE DD genotype implicate a strong possible role in the hypertensive state and in renal damage among north Indians. The study will help in predetermining the timing, type and doses of anti-hypertensive therapy for ESRD patients.

375: Genetic variants of *ACE, NOS3, GSTP1* and *EPHX1* and oxidative stress markers associates with COPD

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Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airflow limitation, permanent distal air-space enlargement and emphysema in the lungs. Oxidative stress is one of the major factors in the pathogenesis. The genetic susceptibility might depend on variations in the associated genes. In a case–control study we investigated the polymorphisms I/D of Angiotensin-converting enzyme (*ACE*), G894T, CA-repeat of endothelial nitric oxide synthase (*NOS3*), I105V, A114V of Glutathione-S-transferase P1 (*GSTP1*) and Y113H, H139R of microsomal epoxide hydrolase (*EPHX1*) along with oxidative stress bio-markers such as malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GPx), Angiotensin-converting enzyme (ACE) and nitrite levels. We examined the association of the genetic variants individually or in combination with disease and their contribution to ACE, Nitrite, MDA, GSH and GPx. The individual distribution of I/D of *ACE* and G894T, CA repeat of *NOS3* did not differ significantly between the two groups. However, the interaction between the genotypes II of *ACE* and GG of *NOS3* was significantly greater (30 vs. 15%, $P = 0.01$) in the controls. The patients were over-represented by the alleles 105V, 114V of *GSTP1* and 113H, 139H of *EPHX1* ($P = 0.002$, 0.001, 0.004 and 0.025, respectively) and the associated haplotypes i.e. 105V–114V and 113H–139H ($P < 0.001$ and $P < 0.001$). Moreover, there was marked over-representation of genotypes combination with 105V + 114V alleles (64 vs. 47%) of *GSTP1* ($P = 0.002$) and the H113H + H139H (27 vs.10%) of *EPHX1* ($P = 0.0001$) in patients. The patients had significantly elevated ACE ($P = 0.05$), MDA levels ($P < 0.001$) and decreased GSH level ($P < 0.001$) and GPx activity ($P = 0.035$). Of note, Subjects having the II and GG genotype combination had lowest ACE activity and highest Nitrite level. The changeover from II to ID to DD resulted into increased ACE activity, whereas the conversion from GG to GT to TT decreased Nitrite levels in both groups. Furthermore, the genotypes, I105V/V105V, A114V/V114V of *GSTP1* and Y113H/H113H of *EPHX1* associated with increased MDA ($P = 0.04$, 0.03 and 0.003) and decreased GSH level ($P = 0.019$, 0.007 and 0.0006), respectively, in the patients; so was the correlation of these bio-markers with genotypes combinations. Gene variants individually or in combinations affecting the function of proteins may alter the level of oxidative stress thereby contributing to the susceptibility to COPD.