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ABSTRACTS

Genomics of complex disorders I

249: Higher order gene–gene interactions: Association of CCL2, CCR5, IL8, AGT, ACE and AGTR1 gene variants with type 2 diabetic nephropathy in Asian Indians

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Diabetic nephropathy (DNP) is an important complication of type 2 diabetes, with 15–60% of patients developing persistent microalbuminuria, progressing to nephropathy and renal failure. Genetic predisposition is a major determinant in the development of renal complications of diabetes. The renin angiotensin system (RAS) and the interlinked inflammatory cascade modulate the progression of renal disease. We hypothesize that gene polymorphisms in the RAS-cytokine pathway, through altered gene expression of inflammatory cytokines, are potential factors that influence the susceptibility to DNP. In the present study, we examined association of 13 single nucleotide polymorphisms (SNPs) within seven candidate loci of RAS cytokine pathway with DNP in Asian Indian subjects.

Research Design and Methods: We analyzed 13 SNPs (rs4311, I/D, rs4343 in ACE, rs5050, rs4762, rs699 in AGT, rs5186 in AGTR1, rs1024611, rs3917887 in CCL2, rs1982073, Tyr81His in TGFB1, rs4073 in IL8, and Del 32 variant in the CCR5 genes) in 495 North Indian subjects with type 2 diabetes [with and without nephropathy (DM)] by using PCR–RFLP assay. We then reconstructed haplotypes by combination of two genes (ACE and CCL2) present on the common locus chromosome 17q. We also assessed the gene–gene interaction among the studied loci using multi-factor dimensionality reduction (MDR) technique.

Results: Among the 13 loci examined, 6 were significantly associated with increased risk of DNP in our population (ACE I/D, $p = 0.004$; AGT, $p = 0.0001$; MCP I/D, $p = 0.006$; IL8, $p = 0.02$; CCR5, $p = 0.0001$). Haplotype analysis (ACE and CCL2) revealed that frequency of haplotype (G-I-T-D-G) was significantly higher ($p = 0.03$) in DNP group as compared to DM group. Haplotypes (A-D-C-I-A, A-D-C-I-G and G-D-C-I-G) conferred significantly ($p < 0.04$) lower risk and were protective. Epistasis observed with MDR showed synergy between two loci rs699 (AGT) and CCR5 del 32 variant further conferring a four fold risk of developing DNP in diabetics.

Conclusion: The RAS cytokine pathway is predisposing with ACE, AGT, CCL2, IL8 and CCR5 gene variants being associated with DNP. Gene gene interaction reveals synergy between CCR5 Del 32 and rs699 variants towards DNP development. Also, a high risk haplotype on chromosome 17q, is associated with DNP in Asian Indians.

250: Common polymorphisms of angiotensinogen (AGT) and susceptibility to hypertension: Prospective implementation of IGVdb

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Introduction: The genes of Renin-angiotensin system are prime candidates of essential hypertension; angiotensinogen (AGT) gene G-6A, T174 M and M235T polymorphisms are well reported potential genetic markers. The aim of this study was to investigate association of these polymorphisms to hypertension in a High Altitude (HA) isolated population and also the prevalence of these three polymorphisms in the 24 indigenous populations of India covered under the IGVdb.

Methods: In a case-control design, 540 consecutive ethnically-matched, high altitude subjects comprising 185 hypertensives and 355 controls were recruited. Genotyping by PCR–RFLP, genotypes combinations and haplotype analyses were performed. In a parallel study same polymorphisms were analyzed on illumina platform, in 552 healthy controls representing 24 indigenous populations covering all corners of India.

Results: The G-6A (rs5049), T174 M (rs4762) and M235T (rs699) polymorphisms does not differed between the cases and controls in this cohort ($p = 0.88586$, $OR = 0.981$, 95% $CI = 0.755–1.274$; $p = 0.928$, $OR = 0.978$, 95% $CI = 0.598–1.763$; and $p = 0.898$, $OR = 1.017$, 95% $CI = 0.78–1.32$, respectively). Three major haplotypes were present with frequency >5%, viz GTM, ATT and AMT. No significant difference was observed in these haplotype ($p = 0.99$, $OR = 1$, 95% $CI = 0.5584–1.7909$; $p = 0.89$, $OR = 1.04$, 95% $CI = 0.596–1.81$; and $p = 0.93$, $OR = 0.95$, 95% $CI = 0.31–2.89$). The common risk haplotype distributions among the 24 Indian populations were overlapping with the cardiovascular mortality data obtained from registrar general of India.
Conclusion: The individual as well as interaction among G-6A, T174M and M235T polymorphisms in combinations or haplotypes does not associate with hypertension in this HA population. The overlapping results of risk haplotype and mortality due to cardiovascular disorders in few ethnic groups do indicate of some genotype-phenotype correlation.

251: Association of Angiotensinogen G-6A and C4072T polymorphisms with hypertension in Mexican population

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In Mexico, hypertension has a prevalence of 30.8% in population >20-years-old. It is a risk factor for myocardial infarction, heart failure, vascular disease, and renal failure. Different genetic variants have been reported to be associated with hypertension, including those in the Angiotensinogen (*AGT*), adrenergic beta-1 receptor (*ADRB1*) and, angiotensin-II receptor type 1 (*AGTR1*) genes. To analyze association of these variants with hypertension in the Mexican population, we conducted a case-control study in 230 cases and 80 controls >65-years-old from Mexico City. We genotyped six SNPs in *AGT*: C-532T (rs5046), G-218A (rs5049), A-20C (rs5050), G-6A (rs5051), C3889T (rs4762) and C4072T (rs699); two SNPs in *ADRB1*: A145G (rs1801252), C1165G (rs1801253); and one variant in *AGTR1*: A1166C (rs5186), using TaqMan technology (AB, USA). We determined allele frequencies, Hardy–Weinberg (HWE) equilibrium, and the odds ratios (ORs) in the dominant, recessive and additive models for each genetic variant. All variants were in HWE with a genotyping call rate higher than 95%. No association with hypertension was shown for polymorphisms in the *ADRB1* and *AGTR1* genes. In contrast, two genetic variants in the *AGT* gene showed a significant association with hypertension in our Mexican population: G-6A (OR 4.64; IC 95% = 1.8–11.5; $p = 0.00008$ in a recessive model), and C4072T (OR 4.42; IC 95% = 1.74–11.2; $p = 0.0009$ in a recessive model). The G-6A *AGT* variant was further genotyped in 4 different Mexican Amerindian populations. Minor allele frequencies in Mayas (0.17), Tepehuanos (0.06), Mixtecos (0.08), and Zapotecos (0.10), were compared with those from the HapMap populations: CEU (0.61), JPT (0.11), and YRI (0.06), indicating significant differences mainly between Caucasian and Amerindian populations. We are currently increasing the number of cases and controls in our study, and will include a genome wide association study to further characterize the genetic bases of cardiovascular disease in the Mexican population.

252: Chronic arsenic exposure impairs macrophage functions and alters inflammatory cytokine profile

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Arsenic is a well-known environmental contaminant, affecting millions of people around the world. More than six million people in nine districts in West Bengal are exposed to arsenic. Skin lesions including premalignant hyperkeratosis, hallmarks of chronic arsenicism, have been reported from the arsenic exposed population in West Bengal. Arsenic is also known to be immunotoxic and immunosuppressive in nature. Owing to the established roles of human macrophages in immune defense, we investigated, whether chronic arsenic exposure could affect adversely the structure and/or function of these major immune cells. The cell adhesion capacity, nitric oxide production and phagocytic capacity were studied in the macrophages of sixty five arsenic exposed and sixty unexposed individuals. Primarily, there was a significant loss of cell adhesion capacity in the macrophages of the exposed individuals when compared to that of the unexposed group. In addition, nitric oxide production as well as phagocytic capacity of the macrophages were significantly (p less than 0.001) reduced in the exposed group. Arsenic exposure also affected the RhoA-ROCK pathway. Rho A-GTP levels have increased while Rho-GDI levels have decreased in the exposed individuals, which in turn influence cell adhesion and phagocytic property of the macrophages. Moreover, chronic arsenic exposure modulated the secretion of various inflammatory cytokines to different extent: IL1B, IL6, IL8 and TNFa were down regulated whereas IL10 secretion was upregulated in the exposed individuals compared to the unexposed. Thus arsenic impairs the immune systems of the exposed individuals. Again it is known that genetic variations play an important role for arsenic susceptibility. Since arsenic exposure could modify the inflammatory cytokine profile, the polymorphisms in the genes coding for these cytokines might well be implicated in arsenic susceptibility. Therefore, we have also undertaken the association study of the polymorphisms of the candidate genes like IL1B and IL10 with arsenic susceptibility. These studies together, would explain the modulations caused by arsenic that leads to arsenic susceptibility at the immunological level.

253: The Cancer Genome: A-ray of hope

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Solid tumors can be broadly classified into two categories. Clinically homogeneous aggressive cancers such as those of the pancreas, biliary tract, liver, etc., exhibit low survival rates and patients succumb to recurrent metastatic tumors following surgery, necessitating development of efficient treatment strategies. On the other hand, clinically heterogeneous cancers including those of the breast, colon, prostate, etc., exhibit variable survival rates and tumor classification is essential for better patient management. It is widely believed that cancer occurs due to genetic instability leading to multiple molecular perturbations in the genome, resulting in activation of oncogenes and inactivation of tumor suppressor genes. In order to understand the molecular basis for the highly aggressive pancreatic cancer, we employed array-based Comparative Genomic Hybridization to identify recurrent copy number alterations (CNAs) that harbor important oncogenes and tumor suppressor genes. We used genome-wide expression profiling and real time RT-PCR to determine the subset of genes located within the CNAs that exhibited comparably altered expression levels. Our results revealed novel candidate oncogenes and tumor suppressor genes involved in diverse pathways including cell motility, apoptosis, mitochondrial oxidative phosphorylation and chromatin remodeling. Studies are underway to characterize the role of these genes in pancreatic cancer. In addition, we have commenced a large scale multi-pronged molecular analysis of esophagous cancer including characterization of known aberrations such as microsatellite instability, Wnt signaling, and determination of p53 and EGFR status and identification of novel aberrations using genomic approaches. Results indicate important molecular differences between adenocarcinoma (common in western countries) and squamous cell carcinoma (the second most common cancer in several population-based cancer registries of the ICMR in India) of the esophagous (ESCC). Genomic microarray studies have identified a novel recurrent amplicon at 10q21 in ESCC. Efforts are underway to identify the 'driver' oncogene(s) resident within this amplicon. Recently, we have initiated a comprehensive characterization of sporadic and familial colorectal cancer occurring in the young, an important problem in India. Results have revealed molecular features in tumors occurring in young patients that are distinct from those occurring in older patients.

254: Impact of genetic polymorphisms in Apolipoprotein A5 on lipid variables and its association with coronary artery disease in Indians

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The incidence of Cardiovascular Disease in India is projected to be the highest in the world, with an increasing number of young Indians falling prey to the disease. Indians worldwide suffer from a triad of high triglycerides, low HDL-C and high LDL-C. Elevated triglycerides (TG) have been established as an independent risk factor for Cardiovascular Diseases (CVD). The apolipoprotein A5 gene (APOA5) has been shown to play an important role in determining plasma triglyceride concentrations. Several Single Nucleotide Polymorphisms (SNP) in the APOA5 gene have been identified. These include $-1131T > C$, $-3A > G$, S19W, and G185C and their rare alleles are associated with elevated plasma TG levels in different populations. No concise data has been published till date on these polymorphisms in the Indian context. Therefore, in the present study we have investigated a case-control study to determine the frequency of these polymorphisms and also to evaluate whether they are associated with an increased risk of Coronary Artery Disease (CAD). Sixty patients with CAD confirmed by coronary angiography (>50% stenosis in one or more arteries and stable or unstable angina) and sixty controls (examined clinically and investigated by electrocardiography to exclude CAD) have been analysed. The frequency of these polymorphisms varies in our population for e.g., G185C, which is associated with CAD in Chinese population, is not polymorphic in our population (total of 120 individuals analysed). At least one of these alleles $-1131T > C$, $-3A > G$, S19W was present in 30% of Indians and the rare alleles are associated with significant elevation in plasma triglyceride levels. The detailed results on the role of the above four polymorphisms will be dealt with during the presentation.

255: TCF7L2 gene polymorphisms do not predict susceptibility to diabetes in tropical calcific pancreatitis but may interact with SPINK1 and CTSB mutations

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Tropical calcific pancreatitis (TCP) is a type of chronic pancreatitis unique to developing countries in tropical regions and one of its important features is invariable progression to diabetes, a condition called fibro-calculous pancreatic diabetes (FCPD), but the nature of diabetes in TCP is controversial. We analysed the recently reported type 2 diabetes (T2D) associated polymorphisms in the TCF7L2 gene using a case-control approach, under hypothesis that TCF7L2 variants should show similar association if diabetes in FCPD is similar to T2D. We also investigated the interaction between the TCF7L2 variants and N34S SPINK1 and L26 V CTSB mutations, since they are strong predictors of risk for TCP. Two polymorphisms rs7903146 and rs12255372 in the TCF7L2 gene were analyzed by sequencing in 478 well-characterized TCP patients and 661 healthy controls of Dravidian and Indo-European ethnicities. Their association with TCP with diabetes (FCPD) and without diabetes was tested in both populations independently using Chi-square test. Finally, a meta-analysis was performed for assessing the overall significance irrespective of ethnicity. We dichotomized the whole cohort based on the presence or absence of N34S SPINK1 and L26 V CTSB mutations and further subdivided them into TCP and FCPD patients and compared the distribution of TCF7L2 variants between them. The allelic and genotypic frequencies for both TCF7L2 polymorphisms, did not differ significantly between TCP patients and controls belonging to either of the ethnic groups or taken together. No statistically significant association of the SNPs was observed with TCP or FCPD or between carriers and non-carriers of N34S SPINK1 and L26V CTSB mutations. The minor allele frequency for rs7903146 was different between TCP and FCPD patients carrying the N34S SPINK1 variant but did not reach statistical significance (OR = 1.59, 95% CI = 0.93–2.70, p = 0.09), while, the TCF7L2 variant showed a statistically significant association between TCP and FCPD patients carrying the 26 V allele (OR = 1.69, 95% CI = 1.11–2.56, p = 0.013). Type 2 diabetes associated TCF7L2 variants are not associated with diabetes in TCP. Since, TCF7L2 is a major susceptibility gene for T2D, it may be hypothesized that the diabetes in TCP patients may not be similar to T2D. Our data also suggests that co-existence of TCF7L2 variants and the SPINK1 and CTSB mutations,

that predict susceptibility to exocrine damage, may interact to determine the onset of diabetes in TCP patients.

256: Contribution of A116C polymorphisms in conferring risk for essential hypertension—a case control study

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Essential hypertension is a common complex condition posing a major risk to life due to associated fatal complications like stroke, coronary artery disease, end stage renal disease etc. Of the several pathways implicated in essential hypertension Renin Angiotensin (RAS) pathway has been studied in greater detail. Angiotensin II Type 1 receptor (AT1R, encoded by the AGTR1 gene) is an important component of RAS system that harbors several polymorphisms of which a silent polymorphism A1166C (cytosine substituted by adenine) in 3' untranslated region is more prevalent. In the present study 638 essential hypertensive and 407 normotensives were studied for demographic parameters. The mean age of the patients was 55.25 ± 9.64 while SBP and DBP were 154.99 ± 21.93 95.11 ± 11.76 respectively. There was preponderance of males (63.0%) among hypertensives with higher incidence of smokers (26.6%) and alcoholics (31.2%) compared to controls (smokers-13.7%; alcoholics-20.14%). The mean BMI (26.73 ± 4.33) showed a significant increase in hypertensives as compared to controls. 63.08% of the cases reported positive family history. Lipid profiles though were slightly elevated in hypertensives did not differ significantly from controls. Analysis of 645 DNA samples (363-hypertensives and 282-controls) for the distribution of A1166C polymorphisms showed significant difference (χ^2 −6.267, 2 *df*, $p \leq 0.05$) between patients and controls with higher frequency of heterozygotes in patients (AA-82.4, AC-16.3, CC-1.4%) as compared to controls (AA-89.4, AC-9.9, CC-0.7%). Stratification of the data showed similar findings among the sexes, cases with positive family history, obesity, habit of smoking and alcohol consumption. When AC and CC genotypes were pooled to compute risk for allele C carriers for hypertension significant variation was observed between male patients and controls (odds ratios of 0.556, CI-0.349-0.885; χ^2 −6.23; $p \leq 0.025$) and hypertensive and control smokers (odds ratios of 0.18, CI-0.039-0.839 (χ^2 −5.802; $p \leq 0.025$).

The present study reveals high risk for AC heterozygotes at AGTR1 locus for essential hypertension. The substitution of adenine to cytosine does not affect the structure of the encoded protein but it may be suppressing cellular AT1R synthesis in the presence of excess angiotensin II-end product of RAS pathway that acts as a vasoconstrictor causing elevation of blood pressure and hypertension.

257: Genetic alterations of the *TP53* gene and their influence on the risk of cervical cancer development among HPV16/18 positive Indian women

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Introduction: Human papillomavirus infection is the major etiologic factor for cervical cancer (CaCx), the foremost common cancer among women of India and other developing countries. Proteolytic degradation of *TP53* gene mediated by viral oncoprotein E6, is one mechanism associated with the pathogenesis of CaCx. Variations within the *TP53* gene might therefore affect disease risk.
Objectives:

1. Profiling of variations within the *TP53* genomic region encompassing the ORF
2. Estimate the association of such variations (alleles and/or genotypes) with CaCx among HPV16/18 +ve individuals
3. To identify haplotypes relevant for disease risk

Material and methods:

1. Sequencing of *TP53* gene [7517561–7519995 of Chr. 17, (−) strand] of 85 CaCx samples (53 HPV16/18 +ve) and 47 normal samples (23 HPV16/18 +ve)
2. Determination of association of various genotypes or alleles with disease from χ^2 or Fisher's exact test
3. Construction of haplotypes using HAPLOVIEW 4.1.

Results: The variations recorded were a 16 bp duplication (intron 3), a novel SNP A11299C (intron 3), SNP C11446G (exon 4) and a *MspI* RFLP at C13494G (intron 6). Analysis restricted to HPV16/18 +ve individuals revealed that the prevalence of intron 3 16 bp duplication failed to differ between cases and controls. A allele at A11299C was significantly ($p = 0.003$) overrepresented among cases (16.7%) than controls (0%). C allele encoding a Pro at codon 72 (C11446G) was significantly higher (Fisher's $p = 0.038$) among cases (57.7%) than controls (34.8%). Absence of *MspI* RFLP or GG at C13494G was significantly overrepresented ($p = 0.001$) among cases (52.3%) than controls (0%). Haplotype analysis based on A11299C-C11446G-C13494G, revealed significant overrepresentation ($p = 0.037$) of C-C-G haplotype among cases (12.4%) than controls (1.2%), while a significantly decreased prevalence ($p = 0.000$) of C-G-C haplotype was recorded among the cases (16.7%) than controls (56.25%).
Conclusion: A11299C polymorphism does not appear to be relevant for CaCx development among HPV positive individuals in contrast to the combinations of variations within exon 4 and intron 6 of *TP53*. Since HPV16/18 infection is the initiating event for CaCx, the study is highly relevant and calls for the correlation of the findings with viral factors such as viral load and haplotypes, predominantly of HPV16, prevalent within the population, to draw useful insights that might lead to development of strategies for efficiently combating HPV infections for prevention of CaCx.

258: CYP1A1, CYP2E1 and GSTP1 polymorphisms and lung cancer risk among Filipinos

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While the putative association between individual genetic polymorphisms and cancer risk remains uncertain, current data strongly suggest that relating it with epidemiological factors such as age, sex,

ethnicity, environmental risk factors and gene–gene interactions makes for a more accurate assessment of the association between genetic polymorphisms and cancer risk. Using molecular epidemiology studies, data can be generated which can lead to the identification of genetically susceptible subgroups in relation to environmental risk factors. Variations in genes such as those of the xenobiotic metabolizing enzymes has been shown to be associated with susceptibility to lung cancer in numerous epidemiological studies. A case-control study was conducted to evaluate polymorphisms in the genes for Phase I and II drug metabolizing enzymes, particularly glutathione-*S*-transferase (GSTP1) and the cytochrome P450 enzymes, CYP1A1 and CYP2E1 and lung cancer among Filipinos. Association between occupational exposure, consumption of red meat, alcohol intake and cigarette smoke exposure were also determined. There were a total of 184 lung cancer cases and 204 age and sex-matched controls genotyped using PCR–RFLP techniques which were subsequently verified through direct sequencing. After univariate analysis using age-matched logistic regression, none of the polymorphic alleles in GSTP1, CYP1A1 and CYP2E1 showed any increased risk nor protective effect for lung cancer among Filipinos.

259: HLA B serological types relevant for HPV16 positive cervical cancer development among Indian women: significant impact on some viral factors

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Introduction: HPV infection is the major etiologic factor for cervical carcinogenesis (CaCx). Only few HPV infected women develop CaCx after a long latent period justifying the role of host and viral factors in HPV induced carcinogenic transformation. HLA class I alleles are relevant host factors as they participate in immune-surveillance against the virus.
Objectives: To identify HLA B serological types that (1) predispose towards or protect against HPV16 related disease development and (2) influence some relevant viral factors (intactness of the viral E2 gene, and HPV16 viral load).
Materials and methods: HLA B serological types were identified by sequence-based typing of exons 2 and 3 of the HLA B gene among 64 HPV16(+) CaCx cases, 35 HPV16(+) and 24 HPV(−) controls, and the HPV16 E2 gene-intactness and viral copy number per 100 ng DNA by Taqman assay. The findings were analysed using test of proportion/Chi-squared trend test and Mann–Whitney test.
Results: Of the 24 identified HLA B serotypes, B*40 was most prevalent among the controls either HPV(−) (50%) or HPV16(+) (37.14%) while B*35 among the cases (32.81%). There was a significant trend of decreasing prevalence of B*51 serotype ($p_{trend} = 0.000$) with increasing severity of lesions from HPV(−) controls (41.67%) to HPV16(+) controls (5.71%) to HPV16(+) CaCx cases (4.69%). Similar observation ($p_{trend} = 0.000$) was recorded for B*81 when compared between HPV(−) controls (8.34%) and HPV16(+) samples (controls and cases; 0%). Overall median HPV16 copy number was significantly higher ($p = 0.000$) among cases (4.1×10^7) than controls (7.68×10^3), or when restricted to samples harboring intact E2. Median HPV16 copy number within cases harboring intact E2 (5.25×10^7) was significantly higher ($p = 0.023$) than within cases with disrupted E2 (4.52×10^3). Median viral copy numbers in samples harboring intact E2 were significantly higher among cases than controls within the serotypes B*07 ($p = 0.040$), B*35 ($p = 0.006$) and B*40 ($p = 0.000$).
Conclusion: The study reflects the interdependent roles of host and viral factors in HPV oncogenesis. HLA B*51 and B*81 serotypes are protective against HPV16 related CaCx development, while none appears to enhance risk of HPV16(+) CaCx. The serotypes B*35, B*40 and B*07 can potentially predispose HPV16(+) individuals to CaCx through high viral loads. Further correlation of such findings with population specific HPV16 lineages and haplotypes is likely to provide useful insights towards developing modalities for combating CaCx.

260: Functional role of Acidic Mammalian Chitinase polymorphisms in atopic asthma

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Background: Recent microarray expression studies in the mice model of allergic asthma suggest Acidic Mammalian Chitinase (AMCase/CHIA) to be a potential candidate gene in asthma pathophysiology. The identification of CHIA gene variants associated with atopic asthma could be useful in identifying individuals at risk for developing asthma.
Objective: To investigate the genetic and functional significance of CHIA polymorphisms with atopic asthma and serum IgE levels in the Indian population.
Methods: Twenty-one SNPs were identified by sequencing DNA from 60 individuals. On the basis of linkage disequilibrium, six polymorphisms were selected and genotyped in unrelated atopic asthmatics ($N = 270$) and controls ($N = 292$); and an independent paediatric cohort (Patients = 150; Controls = 101). The functional significance was demonstrated by reporter gene assays.
Results: The rs3806448G/A promoter polymorphism showed significant association with atopic asthma ($p_{adult} = 0.00001$ and $p_{paediatric} = 0.0002$) and serum total IgE ($p < 0.05$). Also, rs1049132C/T polymorphism was associated with serum total IgE in patients ($p < 0.05$). We generated a two locus haplotype using these promoter SNPs and observed that GT was the most frequent protective haplotype, while AT was found to be the risk haplotype. This was functionally correlated with its transcript level by transient transfection of the reporter constructs containing these SNPs into the human lung epithelial carcinoma cell line A549. We observed that the construct containing the risk promoter haplotype AT resulted in a threefold increase in transcription ($p < 0.0013$) as compared to the protective promoter haplotype GT, suggesting that these promoter SNPs are functionally associated with CHIA gene expression.
Conclusion: This is the first study demonstrating a significant association of CHIA polymorphisms with atopic asthma and total serum IgE. The functional relevance of this study may initiate further research in elucidating the exact role of CHIA in asthma pathogenesis.

261: Genotype and tissue specific effects on alternative splicing of the transcription factor 7-like 2 (TCF7L2) gene in tissues important to type 2 diabetes (T2DM) pathogenesis

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Single nucleotide polymorphisms (SNPs) of the TCF7L2 gene are the strongest genetic risk factors for T2DM, but the variants are intronic and of unknown function. TCF7L2 has multiple alternative splice forms, but no data are available on alternative splicing in tissues playing key role in T2DM pathogenesis. We hypothesized that TCF7L2 SNPs altered T2DM risk by changing expression of alternative splice forms in human tissues involved in T2DM pathophysiology. We isolated total RNA from human subcutaneous (sc) adipose and muscle biopsy samples, and we complemented these studies with HepG2 cells and a commercial total pancreas RNA sample to identify all TCF7L2 splice forms using 5′ and 3′ RACE technique and PCR based cloning. We quantified total and transcript isoform specific TCF7L2 levels in sc. adipose of 78 healthy non diabetic human subjects who were genotyped for SNPs rs7903146 and rs12255372 and who represented a wide range of body mass index (BMI) and insulin sensitivity (SI). We also quantified alternative splice forms in human liver and (HepG2) and adipose cell lines (SGBS) under different in vitro conditions. In adipose we found two alternate 3' ends of TCF7L2 mRNA, one isoform being 262 bp shorter then the other. The shorter isoform was not observed in muscle. We confirmed expression in all tissues of a previously reported 69 bp alternatively spliced exon within intron 3 (exon 3a), and identified a novel 141 bp alternatively spliced exon within intron 4 (exon 4a) expressed only in muscle tissue and HepG2 cells. In muscle, we also observed a novel 235 bp exon formed by alternative splicing of parts of exons 3 and 4. A 73 bp alternatively spliced exon from within intron 13 (13a) was present in all tissues and was present the most abundant isoform in the pancreas. We observed a total of 16 different TCF7L2 alternatively spliced transcripts, Based on screening >80 clones each in adipose, muscle, pancreas and HepG2 cells, we identified 8, 14, 8 and 10 isoforms, respectively. Whereas total TCF7L2 levels in sc. adipose were not correlated with BMI or SI, transcripts retaining exon 13a in adipose were significantly correlated with BMI ($p = 0.01$) and % fat ($p = 0.002$). Furthermore, exon 13a containing transcripts were significantly associated with genotype at SNP rs7903146 ($p = 0.03$). These studies suggest that TCF7L2 intronic SNPs may alter alternative splicing or transcript-specific stability of TCF7L2 in human adipose.

262: Risk of oral leukoplakia and cancer in relation to HPV infection, polymprphisms at *XRCC1* and *TP53* and mutation in *TP53* gene

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Introduction: Apart from tobacco use, oncoproteins in HPV 16/18 have been reported to play important roles in oral carcinogenesis. These oncoproteins can interact physically with different proteins like TP53 and XRCC1, to increase genomic instability and thus the risk of cancer. Polymorphisms in these genes may also affect the interaction between these proteins and HPV oncoproteins and, hence, modulate the risk of diseases in HPV infected individuals.
Aim: In this study we analyzed (a) HPV infection, (b) polymorphisms in *XRCC1* and *TP53* and (c) somatic mutations in *TP53* to estimate the contribution of these factors to oral carcinogenesis.
Methods: Tissue DNA from 274 samples (83 cancer, 91 leukoplakia, 100 controls) were screened for (a) HPV 16/18 infection, (b) polymorphisms in *XRCC1* and *TP53* by PCR-RFLP to estimate the risk of disease independently and jointly and (c) somatic mutations (compared with blood DNA) in exons 5–9 of *TP53* by resequencing to determine the relationship between HPV infection and *TP53* mutation.
Results: (a) HPV infection was significantly associated with increased risk of leukoplakia and cancer (OR = 2.8, 95% CI = 1.2–6.5 and OR = 5.5, 95% CI = 1.6–19, respectively). (b) Genotypes at three polymorphic sites in *XRCC1* did not modulate the risk of diseases independently. Pooled variant haplotypes were overrepresented in overall leukoplakia and HPV non-infected leukoplakia samples (OR = 1.8, 95% CI = 1.2–2.8; OR = 2.2, 95% CI = 1.2–4.0, respectively) but not in cancer samples. The Arg/Arg genotype in *TP53* marginally increased the risk of leukoplakia in overall samples (OR = 2.5, 95%CI = 1.0–6.6) but not in HPV stratified leukoplakia samples. (c) Five mutations in *TP53* gene were detected in five cancer tissue samples and the changes are: C > T (Arg > Cys), C > T (Arg > Trp) and C > T (Arg > stop) at exon 8, G > A (Arg > His) at exon 5 and C > T at intron 9.
Conclusion: The association between variant haplotypes in *XRCC1* and risk of leukoplakia is pronounced in HPV non-infected individuals probably because in infected samples viral oncoprotein could directly inhibit the DNA repair activity of XRCC1 and thus the effect of any polymorphisms in *XRCC1* will not be evident. Although *TP53* Arg/Arg increased the risk of leukoplakia but the finding needs to be validated in a larger sample population. Mutations in *TP53* were observed in both HPV infected and HPV non-infected cancer tissues. Except for the one nonsense mutation, significance of other mutations in *TP53* is yet to be determined.

263: A common genomic variant in 9p21 associated with premature coronary artery disease in Asian Indians

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Coronary artery disease (CAD) is a complex disorder with a broad pathological spectrum and involves both genetic and environmental risk factors. Recent studies have reported the association of multiple common variants, in the 9p21 genomic region, with premature CAD and abdominal aortic and intracranial aneurism in the Caucasian populations. Asian Indians have a high susceptibility to premature CAD and its associated risk factors. The present study was conducted to investigate the association of a 9p21 variant, rs10757278, with premature CAD in Asian Indian population. All participants included in the study were recruited in the Indian Atherosclerosis Research Study (IARS). Genotyping of rs10757278 was performed by a novel method based on real time PCR in 154 affected individuals with strong family history of CAD and 160 healthy controls. The genotyping results were confirmed by sequencing. The G allele frequency was found to be 0.56 in our cohort. The unadjusted odds and the adjusted odds ratio following correction for age, diabetes and hypertension were estimated as 2.19 (95% CI: 1.04–4.64) and 2.15 (95% CI: 1.02–4.6), respectively (observed $p = 0.0098$, empirical $p = 0.016$), in men. The data supported a recessive model for the protective A allele (unadjusted $p = 0.034$, adjusted $p = 0.016$). Our findings are in agreement with the trend observed in other populations

and implicate the 9p21 common variant, rs10757278, in premature CAD in Asian Indians. These results might contribute substantially to the development of the 9p21 chromosomal variants into important markers for the early detection of individuals at genetic risk of CAD.

264: Gilbert's syndrome: Genetic factors and their interactions

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Introduction: Gilbert's syndrome (GS)—hallmark: moderate to high unconjugated bilirubin (UCB) levels—is associated with a dinucleotide TA insertion in the *UGT1A1* promoter. The insertion is neither necessary nor sufficient to cause GS. Hence, the search for other causal and interacting genetic factors is crucial.

Aims: To quantify the (a) extent of association between the TA insertion and GS, (b) involvement of other candidate genes, and (c) interaction between GS and haemoglobinopathies (HB*), since HB* also increases UCB.

Methodology: (1) Unrelated individuals from eastern India were recruited from 4 groups: (1) UCB level in normal range and (a) without HB* ($n = 143$) and (b) with ($n = 41$); (2) UCB level higher than normal and (c) without HB* ($n = 79$) and (d) with ($n = 92$). (2) Resequencing was done for *UGT1A1* promoter, PBREM (3 kb 5′ of *UGT1A1*; known to modulate UCB in response to phenobarbital) and SLCO1B1 promoter. (3) Data were statistically analysed.

Results and Inferences: (1) Age-effect on UCB was not significant in males ($p = 0.73$) and females ($p = 0.265$). (2) Significantly different ($p < 0.0001$) mean UCB levels (mg/dl) in the 4 groups were: (a) 0.36, (b) 0.49, (c) 2.98 and (d) 3.33. (3) The % homozygous for the insertion in *UGT1A1* promoter were: (3.1) 83% in GS cases [Group (c)], *implying that the insertion was not responsible for GS in 17% of cases*; (3.2) only 51% in Group (d), *possibly indicating that the insertion in presence of HB* is deleterious* [supporting fact: among insertion homozygotes with higher than normal UCB level, those with HB* had significantly ($p = 0.03$) higher mean UCB level (4.07) than those without (2.98)]; (3.3) 22% in Group (a), and (3.4) 29% in Group (b), *indicating that 22–29% of TA insertion homozygotes have normal UCB levels.* (4) We detected 2 SNPs (rs57409706, rs10929302) in PBREM; genotype frequencies at both loci different significantly ($p < 0.0001$) among the groups; each with a significant impact (not independent of the effect of the insertion) on UCB. (5) A remarkable structure comprising modal Yin-Yang haplotypes, TG− and GA+ [−, + = without and with insertion; (T, G) and (G, A) are alleles at the two PBREM loci], was observed, with frequencies (%) in the 4 groups: (a) 42,40; (b) 28,52; (c) 5,84; (d) 24,59. Thus, *among HB* individuals, the % of the TG− haplotype is halved compared with normal individuals, with a concomitant increase in* GA+. (6) Variations in SLCO1B1 promoter did not significantly alter UCB.

265: Possible protective role of ARNT gene in low risk for Type 2 Diabetes Mellitus in Raica community of Rajasthan

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The identification of genetic components of Type 2 diabetes (T2DM) has become a real challenge so as to facilitate the disease understanding, its complications, treatment, cure and most importantly prevention. It has already been shown that beta cell dysfunction is a central component of the pathogenesis of T2DM. Across the globe a lot of genes have been studied to understand the pathways behind the disease etiology and one such genes is ARNT. The ARNT gene is crucial for normal development of the embryo and is known to control expression of several other genes. Thus, being a transcription factor this gene serves as a master regulator of many cell functions and is being looked upon as a potential site to integrate genetic and environmental insults responsible for pathogenesis leading to T2DM. ARNT gene is known to be involved in development of abnormalities in insulin secretion and indeed ARNT gene expression has been reported to be decreased in islets from humans with T2DM. The prevalence of T2DM is very low, only 0–0.4% in the Raica community from North–West of India. Thus in the present study the frequency of various ARNT alleles has been studied in the Raica community and other Non-Raica subjects from Rajasthan. The study was conducted in 57 individuals from the Raika community and 75 individuals from Non-Raika communities. DNA samples were genotyped using a PCR–RFLP method. The two groups were also compared for various anthropometric and clinical parameters. Indeed all risk factors studied were very much controlled in the Raica community individuals in comparison to Non-Raika community members. The allele typing results revealed a similar frequency of ARNT alleles in both the Raika and non-Raika subjects. In conclusion, it can be said that the ARNT G1511A polymorphism as such does not seem to be responsible for the very low incidence of T2DM in the Raica community from Rajasthan.

266: A case of balanced reciprocal translocation *t*(3;14)(p12;q12 ~ 13) associated with Bad Obstetric History

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Human chromosomal aberrations are one of the major causes of a disease phenotype. Identification of such aberrations plays a major role in mapping of the disease genes. Here we report a 30year-old male referred for chromosomal analysis due to the Bad Obstetric History (BOH) of the spouse. Cytogenetic analysis had revealed a balanced reciprocal translocation involving chromosome 3 and 14. The karyotype was assigned as 46,XY,*t*(3;14)(p12;q12 ~ 13). Fluorescence in situ hybridizations (FISH) using whole chromosome paints of chromosome 3 and 14 confirmed the translocation. Analysis of the gene content of the break point regions showed that the 14q12 ~ 13 is mapped to a locus for PAX9 gene, a novel member of the paired box-containing gene family. The history showed that his wife had two neonatal deaths one, an anencephalic child and the second child showed absence of stomach bubble and oesophageal atresia. Here the PAX9 gene in association with

the BOH is discussed in detail. As per our knowledge this is the first report involving these balanced reciprocal translocation breakpoints in association with BOH.

267: The Generic-DataSHaPER, a step towards the global harmonization of biobanks

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Sample size calculations taking realistic account of bio-clinical complexity indicate that to comprehensively study the etiological determinants of a complex disease, one ideally needs a minimum of 5,000 cases, and if interest focuses on a gene–gene or gene-life-style interaction, at least 20,000 cases is preferable. But, with rare exceptions, individual biobanks do not contain, and cannot collect, this many cases. In order to optimize the return from investment in biobanking, it is therefore essential to harmonize. Harmonization can be defined as a set of procedures that promote the effective interchange of information and samples between studies, accepting that there may be important differences between them. Biobank scientists have enthusiastically embraced the concept of harmonization, but success demands concrete, scientifically led, actions and effective tools.

A common set of information (including health outcomes, health determinants and physical measures) that may be used by and shared between biobanks has been developed by P3G (the Public Population Project in Genomics) and partner organizations. The Generic-DataSHaPER (Generic Data Schema and Harmonization Platform for Epidemiological Research) supports the construction of baseline questionnaires for general purpose biobanks enrolling middle-aged participants. It covers 60 domains of interest (history of diseases, physical activity, anthropometric measures, etc.) and 250 variables (smoking status, birth location, etc.) and is comprehensive enough to ensure the realization of valid research. The Generic-DataSHaPER is a collaborative project involving experts from more than 25 international biobanks. Its development was guided by a comprehensive review of questionnaires used by population-based biobanks and by the scientific input of international experts. The current version is available on the P3G Observatory website (http://www.p3gobservatory.org/datashaper/explorer.htmIt). Already, eight large population-based biobanks (1 million participants) have used or plan to use the Generic-DataSHaPER in their information collection. Although other key issues—e.g., ethical constraints and quality control assurance—are pivotal to achieving valid sharing of information, the creation of the DataSHaPER is an essential step on the road to effective harmonization. The structure and development of the DataSHaPER will be described, as well as its successes to date and potential uses in biobank harmonization.

268: Evaluation of the frequency of PCOS in patients with breast cancer

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Polycystic ovary syndrome (PCOS) is a heterogeneous, complex genetic disorder characterized by hyperandrogenemia, hyperinsulinemia, insulin resistance, and chronic anovulation. It is the most common endocrine disorder in women of reproductive age with an enigmatic pathophysiologic and molecular basis. Obesity, hyperandrogenism, and infertility occur frequently in PCOS, which mostly have a genetic predisposition, and are features known to be associated with the development of breast cancer risk. This is a case-control study, which compared PCOS frequency in patients with premenopausal breast cancer and the healthy control group with normal mammography in last 6 months. The data analyzed with SPSS 13.0 software using chi-square test. Eleven patients (6.62%) in case group and 16 patients (9.63%) in control group had polycystic ovary syndrome according to their questionnaire. The difference was not significant ($P = 0.645$). In conclusion, there was no relationship between frequency of polycystic ovary syndrome and breast cancer. This might be due to the age of patients with breast cancer in this study, which was mostly over 40. It could be significant if the patients were chosen in lower age for showing more effect of genetic than environment.

269: Evaluation of single nucleotide polymorphisms in Peroxisome Proliferator Activator Receptor-γ (PPARG) and Tumor Necrosis Factor-α (TNF) genes in obese Asian Indians: A population based study

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Background: Obesity is rapidly emerging as a major public health problem in India. It has a strong genetic component and multiple genetic polymorphisms have been implicated. To determine the association of Pro12Ala polymorphism in PPARG gene and Gly308Ala polymorphism in TNF gene with obesity in North India we performed a population based case-control study.
Methods: In an obesity-prone community in Jaipur we performed a population based study using stratified random sampling. Of the 1,400 eligible subjects 20 years or more age we could recruit 1,127 subjects (80.5%). There were 201 (17.8%) subjects with body mass index (BMI) > 30 kg/m2 (Group 1) and 143 subjects with BMI < 20 kg/m2 (Group 2). Clinical, anthropomric, biochemical and nutritional details of these subjects were obtained. SNPs were estimated using PCR–RFLP technique after standardization. Inter-group significance was determined using Chi-square test and logistic regression was used to determine univariate and multivariate associations.
Results: There were more women in Group 1 (119, 59.2%) as compared to Group 2 (62, 43.4%) ($p = 0.004$). In Group 1 the prevalence (%) of truncal obesity (49.8 vs. 19.6%, $p < 0.001$), hypertension (71.1 vs. 46.8%, $p < 0.001$), high total cholesterol (29.9 vs. 18.2%, $p = 0.006$), low HDL cholesterol (28.9 vs. 23.1%, $p = 0.031$), and diabetes (29.9 vs. 12.6%, $p = 0.001$) was significantly greater. There was no homozygosity in the studied SNPs. Heterozygous Pro12Ala polymorphism in PPARG was in 18 subjects (5.2%); 15 (7.5%) in Group 1 and 3 (2.1%) in Group 2 ($p = 0.028$) and heterozygous Gly308Ala polymorphism in TNF in 26 subjects (7.6%), 19 (9.5%) in Group 1 and 7 (4.9%) in Group 2 ($p = 0.115$). Presence of polymorphism of PPARG as well as TNF genes significantly predicted obesity with univariate odds ratio (95% confidence intervals) of 2.25 (1.32–3.84, $p = 0.003$) and 1.48 (1.10–1.99, $p = 0.009$), respectively. These odds ratios remained significant after multivariate adjustments for gender and co-morbidities at 1.74 (1.03–2.93, $p = 0.038$) for PPARG and 1.46 (1.05–2.03, $p = 0.024$) for TNF. Addition of dietary and physical activity variables did not result in significant change in odds ratios.

Conclusions: Asian Indian obese subjects have significant presence of Pro12Ala polymorphism in PPARG and Gly308Ala SNP in TNF genes. The significance remains after adjustment for co-morbidities and dietary and lifestyle variables suggesting direct pathophysiological influence of these genes.

270: Expression-based gene interaction networks for breast cancer

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Breast cancer has become one of the most intensely studied human malignancies in the genomic era. Given the grossly heterogeneous nature of the disease, the notion that a profile comprising of multiple genes, rather than any single gene or other parameter, will be more predictive of tumor behavior is both appealing and reasonable. Therefore, is growing indications that gene–gene interactions may underlie the susceptibility to common human diseases such as breast cancer. It is hypothesized that epistasis is a ubiquitous component of the genetic architecture of common human diseases and that complex interactions are more important than the independent main effects of any one susceptibility gene. In this study we analyzed five independent microarray datasets in order to investigate epistatic interactions that may be the underlying breast cancer subtypes. We present a method to construct gene expression networks using the measure of interaction entropy between gene pairs. Information gain or interaction gain (IG) is described as a measure of the strength of an interaction between attributes and the class label such as case-control status (Jakulin and Bratko 2003 and Jakulin et al. 2003). The decision about the strength of an interaction is based on this p-value of the interaction, visualized as an interaction graph. The results provided five interaction graphs from the five datasets. Functional inter-relatedness of the genes in various molecular events in these five interaction graphs were analyzed by data mining various knowledge-based function annotation and pathway databases like GO, KEGG, HPRD and Reactome. Additionally, we have also attempted to incorporate logic rules (AND, OR, NOT) between the interacting genes using Boolean Logic minimization methods for enhancing our interpretation of the relationship between the genes. Though, the results of our analysis have shown largely non-overlapping interacting genes networks, individually they appear suggest biologically plausible and convincing models for the disease etiology.

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271: A genome-wide association study identifies a novel sarcoidosis disease gene with potential relevance for related granulomatous inflammatory phenotypes

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Sarcoidosis is a complex chronic inflammatory disorder with predominant manifestation in the lung. In the first genome-wide association study (>440,000 SNPs) of this disease, comprising 499 German sarcoidosis patients and 490 controls, we detected a series of genetic associations, the most prominent being with the "SARC2"* gene on chromosome ten. Validation in an independent sample (1,649 cases, 1,832 controls) confirmed the association (SNP rs00*: $P = 3.0 \times 10^{13}$, rs01*: $P = 1.0 \times 10^{-5}$, allele-based test). Extensive fine mapping located the association signal to a region between exons 5 and 14 of SARC2. A common non-synonymous SNP (rs02*, T > C, p.Arg00*-Cys) was found to be strongly associated with sarcoidosis. The GWAS lead SNP and additional risk variants in the region (rs03*, rs04*, rs05*) were in strong linkage disequilibrium with rs02*. The SARC2 protein has complex and essential functions in several biological pathways, including apoptosis and proliferation. We also examined the association of SARC2 with other granulomatous diseases (e.g., Crohn disease, rheumatoid arthritis). Data suggest a potential relevance of SARC2 as a risk gene for phenotypic related disorders and indicate it as a susceptibility locus of general importance.

272: Association of ACE I/D, ADD1 G460W polymorphisms in ischemic and hemorrhagic stroke in North Indian patients

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Introduction: Stroke is a leading cause of mortality and morbidity in India. Genetic predisposition for stroke has been reported world-wide but there is a paucity of genetic study on stroke in North Indian population. Stroke is heterogeneous, multigenic with conventional risk factors playing complex role in pathogenesis. ACE and Adducin variants play a role in essential hypertension which is a known risk factor for stroke. Therefore, this study was aimed to assess the role of genetic variants of angiotensin converting enzyme (ACE) I/D (rs4646994), Adducin (ADD1) G460W (rs4961), in Ischemic and Hemorrhagic stroke in North Indian patients.
Methods: A case control study was performed in 414 subjects (136 Ischemic stoke, 128 hemorrhagic stroke, 150 controls) to evaluate the frequency of polymorphisms of Angiotensin Converting Enzyme (ACE) I/D (rs4646994), Adducin (ADD1) G460 W (rs4961), in Ischemic and Hemorrhagic stroke in North Indian patients. Subjects were genotyped through PCR/PCR–RFLP. Data was analysed by Chi-square test and logistic regression model.
Results: DD genotype of ACE gene was associated with Hemorrhagic stroke [OR = 2.924 CI (1.30–6.54), P = 0.009]. WW genotype of ADD1 gene conferred risk in Hemorrhagic patients [OR = 2.03 CI (1.14–3.16), P = 0.01] but W allele did not modulate the risk in Ischemic stroke [OR = 0.591 CI (0.358–0.975), P = 0.03]. Both W allele and D allele of ADD1 and ACE imparted risk in Hemorrhagic stroke with OR = 2.035 CI (1.14–3.6), P = 0.00, OR = 2.21 CI (1.44–3.40) P = 0.00, respectively.

Conclusion: DD genotype of ACE and WW genotype of ADD1 modulated risk only in Hemorrhagic stroke patients who were more associated with hypertension. However, further studies are required to prove the protective effect of W allele in Ischemic stroke patients.
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273: FTO gene variants are strongly associated with type 2 diabetes but only weakly with obesity in South Asian Indians

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Variants in FTO (fat mass and obesity associated) gene are associated with obesity and type 2 diabetes (T2D) in white Europeans. These associations are not consistent in Asians and there are few reports in South Asian Indians who develop T2D at a much lower body mass index (BMI) than that in the white Europeans. We studied the association of FTO variants with T2D and measures of obesity in South Asian Indians in Pune, India. We genotyped by sequencing, two SNPs rs9939609 and rs7191344, in the FTO gene in 1,453 type 2 diabetes patients and 1,361 controls and a further 961 population based individuals from India. We observed a strong association of the minor allele A at rs9939609 with T2D (OR per allele = 1.26 [95% CI, 1.13–1.40], $P = 3 \times 10^{-5}$). The variant was also associated with BMI but this association appeared to be weaker (0.06SDs; 95% CIs: 0.01–0.10, $P = 0.017$) than the previously reported effect in Europeans (0.10SDs 95% CIs: 0.09–0.12). Unlike in the Europeans, the association with T2D remained after adjusting for BMI (OR per allele for T2D = 1.21 (95% CI, 1.06–1.37); $P = 4.0 \times 10^{-3}$). Similar results were obtained using waist circumference and other anthropometric parameters. Our study replicates the strong association of FTO variants with T2D in South Asian Indians but suggests that the association of FTO with T2D in them might operate through mechanisms other than obesity. This could imply underlying differences between Indians and Europeans in the mechanisms linking body size with T2D.

274: Interleukin 13 gene is a risk genetic factor to asthma in Mestizo-Mexican childhood

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Introduction: Asthma is a complex multifactorial disease caused by environmental influences and at least one hundred of predisposing genes. However, only 14 of them have been replicated in more than ten independent analyses. It is possible that those data have arised from limited statistical power of some studies, but increasing evidences suggest that the results are due to the background genetic differences between ethic groups, which also could explain the differences in the prevalence of the disease and the treatment responses of the patients from several populations. One of the highest replicated association results has been found with single nucleotide polymorphisms (SNPs) in the Interleukin 4 (IL4) and 13 (IL13) genes, however, there are not reports of association with asthma in Mexican patients.
Objective: The present study was undertaken to investigate the association between the IL4 and IL13 SNPs and asthma in Mexican pediatric patients.
Material and methods: Two hundred and forty unrelated asthmatic patients and 408 healthy controls were recruited from three tertiary medical center from Mexico, City. Patients and controls were genotyped to rs2243250 (C/T) and rs2070874 (C/T) on IL4 and rs1800925 (C/T) and rs1881457 (C/T) on IL13 using TaqMan assay. Hardy–Weinberg equilibrium (HWE), haplotypes analyses and statistical significances were performed with FINETTI, HAPLOVIEW and STATCAL software. To evaluate stratification, ten validated admixture markers were included.
Results: Gender ratio male:female in our cases were 1.8:1(64%: 46%). Genotype distributions were in HWE in patients and controls. When IL13 genotypes and allele frequencies were compared between cases and controls a evidence of association was observed to both IL13 polymorphisms: rs1800925 (OR 1.32, IC 95% 1.02–1.7, $P = 0.02$) and rs1881457 (OR 1.38, IC 95% 1.07–1.7, $P = 0.01$). Homozygote genotype TT to rs1881457 SNP increased the risk to asthma (OR 2.02, IC 95% 1.1–3.6, $P = 0.02$). Statistical significances in the TC IL13 haplotype frequency were found (OR = 1.4, IC 95% 1.01–1.8, $P = 0.005$). CC IL13 haplotype were present only in female group. Frequencies of IL4 polymorphisms between cases and controls were similar and diferences were not significant. Frequency of TCTT haplotype including IL4 and IL13 SNPs, was significant higher in cases as compared to controls (OR = 1.29, CI 95% 1.01–1.66, $P = 0.03$).
Conclusions: Our data suggest that IL13 but not IL4 gene is a risk genetic factor to asthma in Mexican pediatric patients.

275: Leptin (LEP) gene: The missing link between the depression, obesity and metabolic disorders

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Background: Epidemiological data have suggested an association between obesity and depression, but findings vary across different studies. Depression and obesity are associated in several ways. First, both are prevalent and disabling disorders influenced both by genetic

and environmental factors. Second, depression, as obesity, seems to be a risk factor for other metabolic disorders like hypertension and cardiovascular disorders. Thirdly, both disorders are associated with less control over appetite, as depressive people tend to eat more or less than their regular energy needs. This fact makes leptin (LEP) gene a probable candidate for common link between both disease states. Aims: Present study is the first attempt which considers the symptoms of depression, metabolic disorders and anthropometric markers in relation to obesity to find possible associations between these. Methods: Blood samples were collected from 150 depressed individuals and an equal number of control subjects without any history of psychiatric disorders visiting Psychiatric unit of BDK Hospital Jhunjunnu and the medical centre facility in BITS, Pilani, Rajasthan. Written informed consent was obtained from each individual or from his/her guardian (in cases of severe depression). The subjects were administrated a questionnaire to inquire about their disease history, occupation, age and socioeconomic status. The anthropometric markers like height, weight, hip and waist circumference were recorded for each subject. DNA was extracted from the PBL's using standard lab procedures. Repeat polymorphisms were detected using PCR–SSLP method. Gels were analyzed by two independent observers for scoring the polymorphism. Data obtained was analyzed with appropriate statistical tools using software like Prism (Graph pad), MEDCCAL and SPSS. Results: A significant difference ($p < 0.05$) was observed in cases and controls for the presence of metabolic disorders and <15 repeats of D7S1875 polymorphism. The values of BMI, WHR, SBP, DBP and FBG were significantly elevated in individuals with <15 repeats of D7S1875 polymorphism when compared with the age matched controls ($p < 0.01$). Conclusions: These results provide first hand evidence that leptin gene may be the possible link between the metabolic disorders and obesity. Data to be presented, will discuss this involvement of leptin gene not only in human obesity and the metabolic disorders but also their associations with the symptomatology of depression.

276: APOE and CAPN gene and risk for the metabolic complications in diabetes

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The diverse incidence of the T2DM across the world and the fact that T2DM is associated with variety of metabolic complications indicates that both the genetic and environmental factors interact to trigger the onset and prevalence of this disease. A high waist to hip ratio (WHR), Body Mass Index (BMI), hypertension and abnormal lipid profiles are taken asrisk factors for T2DM. Infact a gene known to be associated with dyslipidemiais Apolipoprotein E (APOE) gene, which synergistically, in interaction with other genes controls the etiology of T2DM. ApoE, known to play a major role in cholesterol metabolism, is a 299 amino acids long protein and is responsible for transporting lipoproteins, fat-soluble vitamins and cholesterol. This gene is polymorphic with three common isoforms (apoE4 (Cys112 > Arg), apoE3 and apoE2 (Arg158 > Cys)) known. Infact a heterogeneous distribution in the APOE polymorphism is expected in the Indian population, probably due to an admixture of Dravidians (mostly South Indians), Aryans (North Indians), Afro Indians (living in Andaman Nicobar island), Austro-Asiatic and Tibeto-Burmans etc. Another interesting gene conferring a high risk for T2DM in Mexican-Americans is CAPN 10. It has been also recognized as a risk factor for increased serum cholesterol in Chinese population. Present study is an attempt to correlate lipid profile of T2DM patients and evaluate the association of the Apolipoprotein E gene and CAPN10 gene with incidence of T2DM. The study design is a case control type in subjects from different ethnic groups from the state of Rajasthan. The polymorphisms in APOE and CAPN10 gene were detected by PCR–RFLP. In all 80 patients and 89 healthy controls were grouped on the basis of fasting blood glucose levels (FBG) and segregated according to their BMI and WHR. No association was seen in diabetic cases and controls for polymorphism in CAPN10 gene. A significant difference was found for the presence of E*4 allele in females diabetic cases vs control subjects (E*4 vs E*3 allele, Chi: 6.7, $p < 0.01$, Odds Ratio: 2.968, CI: 1.1–1.8). The values for cholesterol and HDL were also found to be significantly higher in diabetic patients with APOE*4 allele ($p < 0.05$). The findings from the present study support a significant role of APOE gene in the etiology of T2DM especially in female subjects. Evaluation of different clinical and anthropometric markers linked to T2DM and their association with APOE genotype will also be presented.

277: Hypervariable polymprphism in SLC8A1 intronic region is associated with cardiovascular traits and serum lipids

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Evolutionarily conserved non-coding regions in human genome may contribute to the regulation of the genes involved in the development of complex diseases. Polymorphisms in these regions could exhibit alternative effects on processes like transcription and splicing. We have screened genetic variation in 16 conserved regions of a candidate gene for cardiovascular disease, SoLute Carrier family eight (sodium/calcium exchanger), member one (SLC8A1). In rats, Slc8a1 has shown to play a role in the development salt-sensitive hypertension. In analyzing cardiovascular disease, patients from two European populations, Estonians (essential hypertension) and Czech (coronary artery disease), we found ten genetic variants (seven novel) located in SLC8A1 conserved regions. We identified an intronic hypervariable polymorphism presented in total nine alleles. The minor variants were represented from singletons to allele frequencies 8.4%. We have performed an association study of the most prevalent alternative variant (a 14 bp insertion) with cardiovascular phenotypes (CAD, MI, heart rate, intima media thickness), blood pressure traits (hypertension, SBP, DBP), and serum lipids. The indel was associated with the incidence of CAD (logistic regression, additive model, $p = 0.005$) and MI ($p = 0.03$), as well as with triglycerides ($p = 0.02$) and intima media thickness ($p = 0.03$). The association with SBP and DBP was detected under recessive genetic model.

278: Negative association of all ten non-HLA single-nucleotide polymorphisms identified in a British genome-wide scan with rheumatoid arthritis in Koreans

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A recent large-scale genome-wide association (GWA) screening for seven common diseases by the Wellcome Trust Case Control Consortium identified 11 single nucleotide polymorphisms (SNPs) for rheumatoid arthritis (RA). In this study, their associations with RA were tested with a Korean population of 1,313 RA-patients and 1,010 non-patient controls, who were genotyped for the 11 SNPs using the MassARRAY® iPLEX system. Among the 11 SNPs, only one SNP in the HLA-class II region was significantly associated with RA susceptibility ($P = 2.66 \times 10^{-35}$) in Chi-square tests for allelic association, and two SNPs (rs6679677 and rs6920220) were not common in Koreans. In order to find new SNP markers covering disease variants in both Asian and European populations, we calculated linkage disequilibrium values (r2) between the non-significant SNPs and their neighboring SNPs using the International HapMap Database and subtracted those in Asians (JPT + CHB) from those in Caucasians (CEU). The ethnic difference in r^2 between the two populations was the largest for rs229484 and the GWA SNP rs743777 on 22q23 (r^2 difference = 0.64). However, genotypes of rs229484 were not significantly associated with RA ($P = 0.096$). Thus, all the ten non-HLA SNPs would not be useful markers for RA susceptibility.

279: Polymorphisms in non-coding region of IFNG gene and their association with atopic asthma

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Background: Interferon gamma (IFNγ) regulates pathological features such as airway inflammation, airway tissue remodeling etc., that are characteristics of atopic asthma.
Objective: To investigate the association of IFNG gene polymorphisms with atopic asthma in Indian population and to elucidate their functional significance.
Methods: Polymorphisms were identified using NCBI database and sequencing 20 unrelated subjects. PCR, Snapshot and GeneScan were used for further genotyping seven polymorphisms in IFNG gene, a (CA)n repeat in intron one and six single nucleotide polymorphisms [(rs2069705, T/C), (rs1861494, A/G), (rs1861493, T/C), (rs2069718, C/T), (rs2069727, A/G) and (rs2069728, G/A)] in family and case-control cohorts. Electrophoretic mobility shift assay was used to elucidate the potential functional role of the SNPs.
Results: A significant association was obtained with A/G (rs18614934) in case control ($p = 0.0006$) and family based ($p = 0.006$) association studies. Also, a five locus haplotypic analysis showed significant association with asthma in case-control ($p = 0.002$) and family based studies ($p = 0.0004$). Moreover our results suggest that the region encompassing the CA repeat, rs1861494A/G and rs2069718C/T is of high priority with respect to asthma pathogenesis. Furthermore, we demonstrate that the wild type allele (A) {A/G (rs18614934)} has stronger affinity for nuclear factor(s) when compared to the polymorphic allele (G).
Conclusion: This study supports the role of IFNG gene polymorphism in genetic susceptibility to asthma. Functional significance of the SNP was partially demonstrated. Identification of the nuclear factor(s) which bind to this locus might further improve our understanding of the IFNG gene regulation and its role in asthma pathogenesis.

280: Physiological effect of human angiotensinogen haplotypes on blood pressure in transgenic mice

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Hypertension is a serious risk factor for myocardial infarction, heart failure, vascular disease, stroke, and renal failure. The incidence of hypertension and complications due to hypertension are even greater in the African-American population. Angiotensinogen (AGT) gene locus is associated with human essential hypertension and over-expression of the AGT gene increases blood pressure in transgenic mice. There are five polymorphic sites (A/G at -6, A/G at -217, T/C at -532, A/G at -793, and T/G at -1,074) in 1.2 kb promoter of the human AGT gene. Variant -217A almost always occurs with -532T, -793A, and -1074T and variant -217G almost always occurs with -532C, -793G, and -1074G. Since allele -6A is the predominant allele (frequency 0.85) in African–Americans, this population can be subdivided into two major haplotypes -6A: -217A (AA); -6A: -217G (AG. The frequency of AA haplotype is significantly increased in African–American hypertensive patients as compared to the AG haplotype. AGT gene is primarily expressed in the liver and reporter constructs containing AA haplotype of the AGT gene promoter have increased promoter activity on transient transfection in human liver cells as compared to the AG haplotype. In order to understand the role of AA and AG haplotypes on human AGT expression in an in vivo situation, we have generated double transgenic mice containing either haplotype AA or AG of the human AGT gene using knock-in strategy at the HPRT locus and human renin gene. Our quantitative RT-PCR analysis has shown that transgenic mice containing AA haplotype have 1.6-fold increase in the AGT mRNA level in the liver as compared to the transgenic mice containing AG haplotype. In addition, Western blot analysis has shown that transgenic mice containing AA haplotype have 30% increase in the AGT protein level in the liver as compared to transgenic mice containing AG haplotype. In addition we show that blood pressure of 3-months-old male transgenic mice containing AA haplotype is increased by 7 mmHg during the day time and 11 mMHg during the night time ($n = 4$ for 4 days) as compared to the transgenic mice containing AG haplotype. To our knowledge, this is the first report where polymorphisms in the promoter of a human gene have been shown to have physiological affect in an in vivo situation.

281: Single nucleotide polymorphisms in homocysteine metabolism pathway genes: association of CHDH A119C and MTHFR C677T with hyperhomocysteinemia

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The incidence of coronary artery disease (CAD) is increasing, especially in developing countries. In India, it has been predicted that deaths due to CAD are likely to be more than any other disease. It is often advocated that a vegetarian diet reduces the burden of CAD. However, in India, where a significant proportion of the population adheres to a vegetarian diet, the incidence of CAD is the highest. This could be due to deficiency of vit.B12, an important micronutrient, sourced only from animal products. Vitamin B12 deficiency can lead to high levels of homocysteine (hcy), an independent risk factor for cardiovascular disorder. We believe that in the background of vit. B12 deficiency, variations in genes involved in hcy metabolism will have greater impact on hcy levels. In this study we genotyped 15 non synonymous single nucleotide polymorphisms (nsSNPs) from eight genes involved in hcy metabolism, in two phases, in an attempt to identify the SNPs that are associated with hcy levels. We also determined the basal frequency of these SNPs in Indian population as a part of the Indian Genome Variation consortium. In the first phase, these nsSNPs were genotyped in 546 individuals. Only two SNPs, MTHFR C677T ($p = 0.001$) and CHDH A119C ($p = 0.009$) modulated the levels of hcy. These two SNPs were then genotyped in an additional 330 individuals. Even after increasing the sample size, MTHFR C677T ($p = 0.002$) and CHDH A119C ($p = 0.007$) were found to be significantly associated with hcy levels. Further, a three way interaction between vegetarian diet and the two polymorphisms was found to significantly elevate the levels of hcy ($p = 0.04$). We also determined the basal frequencies of 13 SNPs in at least 24 subpopulations spread across the country. We found that even amongst the four Indian linguistic groups, Austro Asiatic (AA), Dravidian (DR), Indo European (I.E) and Tibeto Burman (TB), the MAF varied significantly between one or more groups in most of the cases. The frequency of MTHFR 677TT genotype varied throughout the country and in most (29/55) of the populations studied, TT genotype was found to be absent. Interestingly, the TT genotype was present in all the populations of North India (consisting of both I.E and TB linguistic lineage). The risk genotype in choline dehydrogenase (CHDH 119AA) was uniformly distributed throughout the country although the MAF was significantly higher in AA and TB populations as compared to Indo European and Dravidian populations.

282: Interactive effect of Angiotensinogen gene polymorphisms in essential hypertension

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Introduction: Hypertension and associated cardiovascular diseases, alarmingly on rise in India are a global concern. The renin-angiotensin system (RAS) due to well-documented effects that influence vascular tone, cardiovascular remodeling, salt and water homeostasis and cardiovascular diseases has been a logical candidate for evaluation in essential hypertension (EH). The key role of the gene products of the RAS in regulation of blood pressure in humans strongly motivates the study of the gene variants of this system.
Methods: we investigated the *AGT* G-6A (rs5051), T174M (rs4762) and M235T (rs699) polymorphisms individually, in combination and as haplotypes, and their correlation with related phenotypes including systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MAP), plasma renin activity (PRA) and aldosterone levels (PAC) for association with EH.
Results: The genotypes of G-6A and M235T polymorphisms differed significantly between control and patient groups ($P = 0.007$, $OR = 1.9, 95\%\ CI = 1.2–2.9; P < 0.0001, OR = 3.7, 95\%\ CI = 2.3–5.7$, respectively); as a consequence higher frequency of the -6A and 235T alleles was found in hypertensive patients ($P < 0.0001$, each). The GG174TT235MM genotypic combination reflected an OR of 0.43, whereas the remaining genotypes combinations with minimum one to more mutant variants amplified the risk of hypertension to 2.4 times ($P < 0.0001$). Besides, as the number of mutant alleles in a combination increased, SBP, DBP and MAP increased ($P < 0.0001$). It was also observed that 2-locus A/174T, 174T/235T, A/235T and 3-locus A/174T/235T and G/174T/235 M haplotypes served as hypertension risk-predisposing haplotypes; whereas the haplotypes and diplotypes holding -6G and 235M alleles were more frequent in controls and thus these could be protective. Subsequently, hypertensive individuals had higher PAC and ARR than controls, whereas controls showed higher PRA than patients ($P < 0.0001$, each) suggesting that the hypertensive patients likely have low renin hypertension. The -6A and 235T alleles correlated with significantly higher PAC ($P < 0.0001$, respectively).
Conclusion: The -6A and 235T alleles and respective homozygous genotypes were independently associated with hypertension susceptibility. The interaction among G-6A, M235T and T174M polymorphisms in combinations or haplotypes emerged significant. These findings conjoint with significant high PAC and low PRA suggest low renin hypertension in our study population.

283: Epidemiological, cytogenetic and molecular approaches to decipher arsenic susceptibility

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In West Bengal, although a large number of individuals are exposed to arsenic through drinking water, yet, only less than 15–20% of total arsenic exposed individuals showed arsenic induced skin lesions. So it is assumed that genetic variations might play an important role in arsenic induced toxicity and carcinogenicity. We have assessed the arsenic induced health effects and genetic damage in the individuals with and with out skin lesions exposed to same arsenic contaminated water using a number of cytogenetic end points. Genetic and genomic approaches have been adopted to decipher causes of arsenic susceptibility. Comet assay and challenge assay were performed and single nucleotide polymorphisms (SNPs) studies were carried out for a number of genes that may involve the different pathways in arsenic metabolism and detoxification. Results of DNA repair studies through Challenge and Comet assay show that the individuals with arsenic induced skin lesions had suboptimal DNA repair capacity. From the GST's group the homozygous null gene frequency of GSTT1 and GSTM1 genes and SNPs of some other GST genes were also studied. SNPs of TP53 gene, human purine nucleoside phosphorylase (PNP) and ERCC2 genes were also analyses. Distribution of homozygous GSTM1 null genotype was significantly higher in the no skin lesions group indicating a protective role of GSTM1 null in the no skin lesions individuals. On the other hand the individuals with TP53 codon 72 Arg/Arg genotype is over represented in the skin lesions individuals indicating that this genotype is more susceptible to arsenic induced toxicity. Lys/Lys genotype in the ERCC2 polymorphism was almost five fold over represented in the arsenic induced hyperkeratosis skin lesions group when compared to no skin lesions group. We also showed that risk genotypes of ERCC2 and TP53 SNPs were positively correlated to genetic damage in having significantly higher chromosomal aberrations compared to the referent genotypes. Thus

the above results indicate that the sub-optimal DNA repair and the genetic variations are responsible for arsenic induced toxicity and carcinogenicity.

284: Association of Apolipoprotein A4 polymorphisms Asn147Ser and Thr347Ser with type 2 diabetes in an Asian Indian population

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Aim: The APOA4 gene has been implicated in the development of dyslipidemia in Type 2 diabetes patients. The objectives of the present investigation was to examine the relationship of two polymorphisms located in the exon 3 of the Apolipoprotein A4 gene (APOA4), Asn (N) 147Ser(S), A > G (rs 5104) and Thr(T) 347Ser(S), A > T(rs 675) in the Asian Indian population.
Methods: The study group comprised of 762 type 2 diabetes and 536 Normal Glucose Tolerant (NGT) subjects chosen from the Chennai Urban Rural Epidemiological Study, an ongoing population-based study in Southern India. The two polymorphisms were genotype using PCR–RFLP method and confirmed by direct sequencing. The genotype frequencies were estimated by using Chi-square analysis. The Odds ratio was estimated using Logistic regression Analysis from SPSS Version 10.0.
Results: 62% of the type 2 diabetes patients had the Asn/Ser genotype compared with 53.8% of Normal Glucose Tolerant subjects (NGT), $p = 0.01$. Logistic regression analysis of the Asn147 Ser polymorphism showed the odds ratio (adjusted for age, body mass index [BMI]) was 1.62 (95% CI: 1.92–2.22, $p = 0.002$) for the Asn/Ser genotype when compared with the Asn/Asn genotype. In case of the Thr347Ser polymorphism, 27.2% of the type 2 diabetes has Thr/Ser genotype when compared with 35.6% of the Normal glucose Tolerant subjects (NGT), $p = 0.01$ showing a protective effect towards type 2 diabetes. Logistic regression analysis of the Thr 347 Ser polymorphism showed the odds ratio (adjusted for age, BMI) was 0.606 (95% CI: 0.451–0.815, $p = 0.001$) for the Thr/Ser genotype when compared with the Thr/Thr genotype.
Conclusions: The two polymorphisms, Asn147Ser and Thr347Ser were associated with Type 2 Diabetes in Asian Indian subjects. The Asn/Ser genotype of the Asn147Ser polymorphism confers 1.62 times higher risk for type 2 diabetes, whereas the Thr/Ser genotype of the Thr347Ser polymorphism confers 0.606 times lower risk for developing type 2 diabetes in this population.

285: Genome-wide association study with blood pressure traits

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Hypertension is a common disease affecting 25% of the adult population. Different studies have suggested that approximately 33–66% of the inter-individual variance in BP level is heritable. In order to search for novel genetic variants associated with BP levels, we conducted a genome-wide association study (GWAS) for three blood pressure traits (SBP, DBP and HYP) in the KORA (Kooperative Gesundheitsforschung in der Region Augsburg) S3/F3 epidemiological cohort ($n = 1{,}644$) recruited from a general population in Southern-Germany. Using a three stage study design, we identified two new loci (at 16q23 and 17p13) associated with BP traits and replicated these associations in two European populations (Germans, Estonians). In a third population (British) we obtained consistent effect signs but borderline P values. The minor variants of these SNPs reduce BP and therefore, protect from the development of hypertension (joint analysis, $n = 1{,}900$ hypertensives/4,365 normotensives: $P = 5.41 \times 10^{-8}$, OR = 0.73 and $P = 7.42 \times 10^{-7}$, OR = 0.65, respectively). The identification of two novel loci encourages further research to clarify the functional basis of the identified associations.

286: Identification of unique primary and secondary Glioblastoma Multiforme (GBM) specific networks and molecular connections using protein–protein interaction tools

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Gliomas of astrocytic origin have been classified by WHO into tumors that arise de novo (primary GBM) and those that manifest progression from a low grade tumor to a highly invasive, metastatic type (secondary GBM). These clinical differences are also accompanied by a set of distinct molecular and genetic abnormalities. EGFR amplification and PTEN deletions are hallmarks of primary GBM, whereas TP53 and PDGFRB mutations are common to secondary GBM. The advent and extensive use of high throughput genomic and proteomic tools on a genome wide level allows individual's gene expression to be analyzed on a global scale. Early exercises of these tools have delineated several cellular perturbations associated with oncogenesis. Recently a combinatorial approach of differentially regulated genes and protein interaction network of these gene products have provided valuable information of molecular connections in different pathways which go awry in the diseased condition. We have used this approach to construct cellular network characteristic of primary and secondary GBM and identified novel molecular connections in these two types of GBM. Clinically and experimentally validated genes that showed specific up regulated expression pattern in primary and secondary glioblastoma (58 and 22 genes respectively) were used as the input and subjected to Systems biology analysis to construct molecular interaction network of proteins using APID

interaction database and Cytoscape software. This generated a compiled molecular network of primary and secondary GBM specific proteins. Careful dissection of the functional modules, important nodes and connections allowed identification novel intermediary molecules (5 in primary and 2 in secondary GBM network) that have potential to be assessed for their relevance in gliomagenesis and prognostication. These genes are functionally important centered around the biological processes such as signaling by tyrosine kinase receptors, extracellular matrix remodeling and cell proliferation in primary GBM and around cell cycle regulation, apoptosis and evasion of immune response in secondary GBM. These molecules can be combined with the existing molecular signatures to predict the outcome of glioma and can also be compared with other forms of glial disorders to differentiate between confusing tumors types by combining mutational and epigenetic information.

287: Somatic DNA amplification as a phenotype in cancer

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Cancer is known to have a complex etiology, with both inherited and somatic components. Recent studies have yielded promising results using SNPs as markers in associations studies, detecting associations between certain SNP alleles and susceptibility to a variety of cancer types. However, bearing in mind that somatic copy number changes are a hallmark of cancer, we have conducted a study treating somatic copy number lesions (representing the cancer genome) at each gene as an phenotype, with inherited SNP alleles (representing the germline genome) as predictors. We have analyzed 474 lung and breast cancer samples. For each of these samples, we possess Affymetrix 250K SNP array data for both tumor DNA and matched normal DNA. Our analysis reveals abundant cis associations between inherited variants and somatic amplification of certain genes, including well-known oncogenes. We also report some evidence for trans associations. This study demonstrates that somatic amplification may be treated as an intermediary molecular phenotype in cancer, and that the germline genome and tumor genome may be simultaneously interrogated in the patient to gain additional insight into inherited cancer susceptibility.

288: A comprehensive approach towards analysis of complex disorders: Tropical calcific pancreatitis as a model

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Chronic pancreatitis (CP) is an inflammatory disease of pancreas characterized by irreversible destruction of exocrine mass and progressive endocrine failure leading to pancreatic insufficiency and diabetes. It has been proposed that CP occurs due to activation of trypsinogen within pancreas before being secreted into the duodenum. A non-alcoholic type of juvenile and severe form of CP with uncertain etiology, specific to tropics is known as tropical calcific pancreatitis (TCP). We established the genetic basis of TCP and showed it to be different from the West. A founder mutation N34S in SPINK1 gene was detected in majority of patients in contrast to cationic trypsinogen mutations in Caucasians. To understand the etiopathology of TCP a comprehensive study on genomic and proteomic front was designed. Several candidate genes were screened for associated variants, hypothesizing their role in the initiation of pancreatitis by gain of function mutations leading to premature activation of trypsinogen. Variants in the signal peptide region of cathepsin B (CTSB) were found to be associated with TCP and proposed to be the second candidate gene. No association was seen with genes such as anionic trypsinogen (PRSS2), Transcription factor 7 like 2 (TCF7L2) and regenerating islet-derived protein 1α (REG1A) selected for their role in causation of exocrine damage, diabetes and stone formation, respectively. As there are no reports on systematic study of gene expression in CP patients, we conducted microarray analysis using cDNA and oligonucleotide (Affymetrix) platforms on pancreatic tissue samples from TCP patients and normal individuals as per International guidelines and identified several genes to be differentially regulated. Classification of genes based on Gene Ontology descriptions revealed down-regulation of genes coding various digestive enzymes corroborating various clinical features of TCP. Pathway analysis revealed pathways like cell adhesion molecules (CAM), inositol 1,4,5-triphosphate receptor (IP3R), Mitogen activated protein kinase (MAPK), etc., to be involved, suggesting an ongoing apoptosis and necrosis in the patient tissue. Real-Time PCR on selected genes validated the results obtained from microarray experiments. Proteomic profile of the pancreatic tissues from these patients identified about 35 differentially regulated proteins. Finally, through systems biology approach, we attempted to network various genetic and non-genetic factors involved in the pathogenesis of TCP.

289: Association of the pituitary growth hormone and its receptor gene polymorphisms with premature coronary artery disease in Asian Indians

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In view of the inadequacy of our knowledge, till date, on the exact number of genes and the quantum of their effect on the etiopathology of cardiovascular disease (CVD), it becomes imperative that further investigations on novel genetic risk factors are conducted on the predisposed yet previously untested populations like the Asian Indians. The present study was undertaken to investigate the putative association of the polymorphisms in the pituitary growth hormone (GH1) and its receptor (GHR) genes with premature coronary artery disease (CAD), the most common form of CVD, in Asian Indians. The study participants, selected from the ongoing Indian Atherosclerosis Research Study (IARS), consisted of 178 patients of premature CAD and 151 healthy controls. Sequencing of the promoter region of the GH1 gene revealed 8 novel sequence variants in our cohort. An AGA promoter haplotype was found to be significantly

associated with CAD. The unadjusted and the adjusted odds ratio (OR) after correction for age, gender, diabetes and hypertension were estimated to be 2.64 (95% CI: 1.24, 5.6, p = 0.012) and 3.77 (95% CI: 1.35, 10.50, p = 0.012), respectively, for this association. The presence of the exon 3 deletion in the growth hormone receptor gene (GHRd3) was detected and quantitated by real time PCR assays. The absence of GHRd3 was found to be significantly associated with CAD in patients with a history of hypertension (p = 0.018). The data supported a dominant genetic model for GHRd3 allele with unadjusted and adjusted OR of 0.51 (95% CI: 0.29, 0.90, p = 0.018) and 0.40 (95% CI: 0.19, 0.85, p = 0.014), respectively, after correction for age, gender and diabetes. Our results implicate GH1 and GHRd3 polymorphisms in premature CAD in Asian Indians. These novel findings might help in opening new avenues towards understanding of cardiovascular disease biology from the perspective of growth and development as well as assist in the evolution of important markers for the early detection of genetic predisposition to CAD.

290: Association of Xenobiotic metabolizing enzymes genetic polymorphisms with esophageal and gastric cancers in Kashmir valley: Influence of smoking and consumption of salted tea

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Introduction: Xenobiotic metabolizing enzymes are involved in the detoxification of many potential carcinogens and appear to play a critical role in the protection from the effects of carcinogens. Kashmir valley has elevated incidence rate of esophageal (EC) and gastric (GC) cancers. Several environmental and host genetic factors have been suspected for development of EC and GC. Therefore, this study was performed to assess the association of genetic variants of xenobiotic metabolizing enzyme genes with susceptibility of esophageal and gastric cancers in Kashmir valley.
Methods: A case control study was performed in 381 subjects (108 EC, 72 GC and 201 healthy subjects) to analyze association of polymorphisms in GSTM1null, GSTT1null, GSTP1 313ile/val, GSTM3 intron6 3 bp deletion,CYP1A1 6235T > C and CYP2E1Rsa1-1091C > T genes with susceptibility of EC and GC as well as their interaction with smoking and high consumption of salted tea. All subjects were genotyped through PCR/PCR–RFLP. Data was analyzed using chi-square test and logistic regression model.
Results: GSTP1313 val/val and CYP2E1c1c2 genotypes imparted risk for EC (OR = 2.966, P = 0.034; OR = 2.802, P = 0.015) while individuals with GSTT1null and CYP2E1c1c2 genotype were at higher risk of GC (OR = 2.492, P = 0.044; OR = 2.015, P = 0.032). Smokers were at higher risk for developing EC and GC (OR = 21.465, P = 0.0001; OR = 16.338, P = 0.0001). Similarly, higher consumption of salted tea was also associated with increased risk of EC and GC (OR = 18.179, P = 0.001; OR = 16.338, P = 0.0001). Smokers with GSTT1null and high salted tea consumers with GSTM1null genotypes were at increased risk for GC development (OR = 3.213, P = 0.047; OR = 18.535, P = 0.009).
Conclusions: GSTP1 val/val, GSTM3AB and CYP2E1c1c2 genotypes modulate risk of EC; while GSTT1null and CYP2E1c1c2 genotypes impart risk for GC. GSTT1null and GSTM1null genotypes enhance risk of GC in smokers and high salted tea consumers in Kashmir.
Acknowledgment: The study was supported by a research grant from Indian Council of Medical Research, New Delhi.

291: Association of polymorphic markers in candidate genes with quantitative precursors of Coronary Artery Disease: A study in an isolated population group of India

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Aim: To map genes that control ten quantitative precursors of coronary artery disease (CAD), with the overarching goal of genomic dissection of CAD. The quantitative traits (QTs) selected were apolipoprotein B [ApoB], C-reactive protein [CRP], fibrinogen [FIB], homocysteine [HCY], lipoprotein a [Lp(a)], total cholesterol [CHOL-T], HDL-cholesterol [CHOL-H], LDL-cholesterol [CHOL-L], VLDL-cholesterol [CHOL-V] and triglyceride [TG].
Methodology: We focused on an isolated population—Marwari, known to have a high prevalence of CAD. Members ($n = 459$) of 45 extended families, each ascertained through the presence of at least two first-degree relatives with CAD were recruited. A total of 209 SNPs in 31 genes selected from a relevant biochemical pathway were assayed. From the family data, a subset of 155 unrelated individuals was identified. After adjusting for demographic, anthropometric, life-style and behavioral variables, using a stepwise regression analysis, ANOVA was performed to test the equality of mean values of adjusted QTs among genotypes at each SNP locus. For each locus for which statistically significant difference was detected for any QT, a quantitative transmission-disequilibrium test (qTDT) was performed using data on nuclear families (327 individuals in 144 families).
Results: Statistically significant results found, on the basis of ANOVA and qTDT analyses, for the various QTs and SNPs were: (1) ApoB with *NFKB1*: rs230521 (Intronic) ANOVA p-value (p) = 0.007, qTDT p-value (p^*) = 0.024; and, rs1005819 (Intronic) $p = 0.01$, $p^* = 0.02$. (2) FIB with *NFKB1*: rs4648004 (Intronic) $p = 0.04$, $p^* = 0.002$. (3) FIB with *FGB*: rs4220 (Missense, K > R) $p = 0.009$, $p^* = 0.04$, (4) Lp(a) with *SCARB1*: rs4765180 (Intronic) $p = 0.02$, $p^* = 0.01$. (5) CHOL-H with *SELE*: rs5361 (Missense, R > S) $p = 0.01$, $p^* = 0.03$. (6) CHOL-H with *MYD88*: rs7744 (3′UTR) $p = 0.02$, $p^* = 0.04$. (7) CHOL-V with *SELP*: rs3917744 (Intronic) $p = 0.02$, $p^* = 0.005$. (8) CHOL-V with *CD14*: rs4914 (Synonymous) $p = 0.002$, $p^* = 0.04$. No statistically significant results were found for CRP, HCY, CHOL-T, CHOL-L and TG.
Conclusion: *SELE*, *SELP*, *CD14*, *MYD88*, *SCARB1*, *FGB* and *NFKB1* are genes that impact on levels of quantitative precursors of CAD in Marwaris. The K > R change in *FGB* increases fibrinogen level. The R > S change in *SELE* is 'damaging' (SIFT score = 0.01) and increases thrombin generation. Higher density saturation studies are required to identify causal SNPs that may be in linkage disequilibrium with the remaining 7 SNPs.

292: CYP17A1 (T-34C), CYP19A1 (Trp39Arg), and FGFR2 (C-906T) polymorphisms and the risk of breast cancer in South Indian women population

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Breast Cancer is initiated by exposure to endogenous and exogenous estrogens. Genes involved in biosynthesis of estrogens and growth

factor receptors play an important role in breast cancer development. A case ($n = 250$)—control ($n = 500$) study was undertaken to investigate the role of Single Nucleotide Polymorphisms (SNP's) in CYP17A1 (T-34C), CYP19A1 (Trp39Arg) and FGFR2(C-906T). Genotyping was done using Taqman Allelic discrimination assay for CYP17A1 (T-34C) and FGFR2 (C-906T) and PCR-CTPP for CYP19A1 (Trp39Arg). There was a significant association of heterozygous (TT/CC) genotype of CYP17A1 gene with the risk of developing breast cancer (OR = 0.68, 95% CI: 0.49–0.96). And the same genotype of the CYP17A1 gene was significantly associated with deceased risk in postmenopausal women (OR = 0.56, 95% CI: 0.35–0.89) (p = 0.015). CYP19A1 (Trp39Arg) is a rare polymorphism, all the cases were homozygous for wild type Trp allele (100%); in controls 99.2% were homozygous for wild type and 0.8% was heterozygous. We are unable to detect the variant form of the CYP19A1 gene in south Indian women population. There was no significant association between the risk of breast cancer and FGFR2 (C-906T), which is supposed to be a newly identified gene linked with breast cancer incidence in western population. These results suggest that CYP17A1 TT/CC genotype was associated with decreased risk for breast cancer especially in post menopausal women. CYP19A1 (Trp39Arg) and FGFR2 (C-906T) have no role to play with breast cancer risk in South Indian women population, further studies with more cases and control are needed to evaluate the role of these genes risk in South Indian women population.

293: Clinical, biochemical and genetic analysis of Leigh syndrome patients with atypical presentation

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Leigh syndrome is a progressive neuro-metabolic mitochondrial disorder usually presenting in infancy or early childhood with varied clinical features and evidence of genetic heterogeneity. Leigh syndrome in India has hardly been explored and hence, we attempted to understand its clinical, biochemical, imageological, and genetic basis in 165 patients from South India. All the patients were infants, presented in acute life threatening condition and responded dramatically towards thiamine supplementation. Electron microscopic and histochemical analyses revealed the structural and functional defects in mitochondria. All the investigations, suggested a different phenotype of Leigh syndrome in Indians.

To investigate the genetic basis of this phenotype, complete mitochondrial genome and nuclear genes encoding components of respiratory complexes were screened in the patients and 94 normal infants. Based on bioinformatics analysis using Clustal W, SIFT and PolyPhen, seven mutations in different genes were investigated using in vitro assays to understand likely mechanism responsible for the phenotype. Effect of mitochondrial mutations were analysed by generating cybrids while SURF1 mutations were analyzed using wild and mutant SURF1 cDNA constructs followed by preparation of stable clone in COS-7 cells. Two mutations, G6036A (G45S) in MT-CO2, found in three patients and (exon 9, C > T) P298L in SURF1, present in 6 patients were predicted to affect the conserved residues and affect the stereochemical property of the protein. Both mutations showed ~50% decrease in Complex IV activity suggesting that defect in complex IV may be one of the major causes for such a phenotype. Since majority of patients responded to thiamine supplementation, estimation and comparison of thiamine level in blood samples of patients and randomly selected normal individuals from same geographical region suggested that thiamine deficiency alone could not cause the disease but may add to the severity of the phenotype. To conclude, our study involving the largest cohort of LS patients gives an idea about the atypical presentation of Leigh Syndrome patients in Indian population and shows a variable genetic basis. It also suggests that thiamine deficiency could be an additional factor, influencing the phenotype in the presence of genetic abnormality. In addition, it also alerts the clinicians towards the importance of thiamine supplementation for such a phenotype, as timely thiamine supplementation can save several precious lives.

294: Multiple HLA-DR3 haplotypes associated with autoimmunity in North Indians

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The aim of the study was to define HLA-DR3 positive extended haplotypes associated with susceptibility to Type 1 diabetes in North India. We evaluated multiple SNPs and microsatellites located between HLA-A and DQB1 locus in the MHC region in 145 North Indian T1D patients. The MHC halotypes were deduced from family pedigrees and compared with those prevalent in healthy Indian population. A comparison of frequencies of HLA class I alleles among T1D patients and healthy controls showed a significant increase in HLA-A*02, A*26, B*08, B*50 and B*58 in the patient group. Similarly, analyses of class II genes revealed a strong association of DR3-DQ2 among the patients (75.9% vs. 14.6% in controls, p = 7.53 E-11). Further molecular analyses revealed that there are multiple DRB1*03 positive haplotypes (predominantly B8-DR3, B50-DR3 and B58-DR3) that are associated with T1D in the Indian population. These haplotypes differ significantly from the classical Caucasian AH8.1 (HLA-A1-B8-DR3), associated with several autoimmune diseases. The Caucasian AH8.1 is rare in the Indian population and has been replaced by a variant AH8.1v and other DR3 positive haplotypes, A26-B8-DR3 (AH8.2), HLA-A24-B8-DR3 (AH8.3), A3-B8-DR3 (AH8.4), A31-B8-DR3 (AH8.5), A2-B8-DR3 (AH8.6), A11-B8-DR3 (AH8.7) and A33-B8-DR3 (AH8.8). Among these, the AH8.2 is the most common haplotype and represents 43% of the total B8-DR3 haplotypes in this population. The Indian B8-DR3 haplotypes differ significantly from Caucasian AH8.1 at multiple loci. For example, the Indian haplotypes have HLA-Cw*0702, HLA-DRB3*0202, HSP70-21267A, TNFA-308G, TNFa 105, Bf-F, C4A-1, MIB 352 as compared to Caucasian HLA-Cw*0701, HLA-DRB3*0101, HSP70-21267G, TNFA-308A, TNFa 99, Bf-S, C4A-0, MIB 350. These differences suggest that B8-DR3-DQ2 haplotypes in the Asian Indian population might have originated independently of Caucasian AH8.1 selectively through recombination and multiple mutations. Among these B8-DR3 haplotypes, a significant association of AH8.2 (p = 2.91 E-06), AH8.3 (p = 2.01 E-05) and AH8.6 (p = 1.13 E-07) was observed with T1D. These findings have important implications in understanding disease associations along with their evolutionary significance.

295: Mutations in the APC gene in families with Familial Adenomatouos Polyposis (FAP)

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Colorectal cancer has been recognized as one of the more common causes of early death due to malignancy worldwide. About 20% of all colon cancer cases are thought to be hereditary. Two well defined forms of hereditary colon cancer are familial adenomatous polyposis coli (FAP) and hereditary non-polyposis colon cancer (HNPCC). Germline mutations in the tumour-suppressor APC gene, localized on 5q in 1991, are associated with FAP. The vast majority of these mutations are nonsense or frameshifts resulting in non-functional, truncated APC protein products.

The present study was carried out to characterize the causative genetic mutation in several medium sized Malaysian families affected with FAP. Mutation screening was performed using the SSCP analysis technique, after which the exons showing mobility shifts (variant bands) were sequenced to determine the nature of the sequence change. SSCP results showed mobility shifts various exon, including 8, 11, 13 and 15. Sequence analysis revealed polymorphisms (exon 15) and patient specific mutations (in exons 11, 13 and 15) which have proved valuable for presymptomatic diagnosis of at-risk family members.

296: Gene expression profiling of the hepatic transcriptome in the presence of TNF α

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Diabetes mellitus, often simply termed Diabetes, is a syndrome characterized by disordered metabolism and high blood sugar. It is caused due to low levels of insulin hormone or from abnormal resistance to insulin in its target tissues. World Health Organization estimates that India will alone have 79.4 million diabetic patients in 2,030. One of its major form Type 2 diabetes, is often associated with obesity, hypertension, elevated cholesterol and metabolic syndrome. Changes in life style, such as consumption of high-calorie diet and lack of exercise, have increased the global prevalence not only of diabetes but also of obesity. Type 2 diabetes is characterized by insulin resistance in target tissue, occurs due to several reasons and one of them being the proinflammatory cytokine, TNFα. It is also known as the link between diabetes and obesity. High levels of TNFα interfere with insulin signaling to cause the effect and to further investigate into the situation, gene transcription profiling was examined in control and TNFα treated HepG2 cells. Results indicated that TNFα could significantly alter the expression of a significant number of genes that were identified to be related to lipid and fat metabolism on one hand and to immunoglobulin receptor activity and IgE binding thereby on the other thereby indicating global dysregulation of fat metabolism and compromise in immune defense mechanism(s) within the hepatocyte by TNFα. Pathway analysis revealed 'biosynthesis of steroids' to be most effected. All these indicate TNFα to be significantly altering the transcriptome profiling within HepG2 cells with genes involved in lipid and steroid metabolism being the most favoured and this could explain one of the underlying mechanisms of TNFα action in the liver.

297: Differentially expressed transcripts in the adipose tissue of 'sumo rats' (WNIN/Ob)

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The etiology of obesity and associated disorders involve very complex gene–gene and gene–environment interactions, making it a formidable challenge to study their molecular mechanisms in human. Therefore, the best way to study the pathophysiology of these disorders is to employ various animal models of genetic and non-genetic animal models of obesity. A rat model of obesity has been developed at the National Institute Nutrition, Hyderabad. These 'sumo rats' (WNIN/Ob), originally identified from an inbred Wistar rat line, attain bodyweight more than twice of their lean littermates, are also hyperphagic, euglucaemic. They also show hyperinsulinaemia, hypertrigleridaemia and hypercholerolaemia characteristic to human obesity. To identify the transcripts that are over or under expressed in the adipose tissue of these rats, we carried out forward and reverse subtraction hybridization PCR using the RNA isolated from the adipose tissue of the sumo rats and their lean littermates. Genes identified to be overexpressed in the adiopose tissue of these obese rats include members from a wide range of gene families like lipid and carbohydrate metabolism, electrolyte transporters, general and specific transcription factors, signal transducers etc. Some of these genes were previously known to be over expressed in obesity whereas others are so far not implicated in the development of obesity. Functional characterization of these genes are in progress using siRNA and transient over expression studies.

298: Attenuation of hepatic insulin sensitivity by TNFα

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Type 2 diabetes is almost invariably associated with obesity and the adipose tissue that was originally identified as an inert storage organ, is now appropriately classified as an endocrine organ. Several factors have been identified as being released from the adipocytes and their circulatory levels have been found to correlate proportionately to the adipocyte mass and the associated status of insulin resistance. Tumor necrosis factor alpha (TNF-alpha) is one such factor that is increased under these conditions and it inhibits insulin signaling by interfering at several points of the signaling cascade. Of the several insulin target tissues, the liver is critical in maintaining circulatory glucose levels through hepatic glucose output and alterations in this phenomenon aggravates an already existing hyperglycemic status as observed in obese diabetics. Using the human hepatoma (HepG2) cell line, we studied the role of TNF alpha in the regulation of this pathway and determined the molecular mechanisms underlying the effect(s) of TNF-alpha in insulin action on hepatic gluconeogenesis. TNF-alpha significantly attenuated insulin induced inhibition of the expression of gluconeogenic enzymes and hepatic glucose production. Since the transcription factor, Foxa2 has in part been implicated in the regu-

lation of gluconeogenic gene's transcription, we studied the effects of TNF-alpha and/or insulin on its cellular status in the hepatocyte. Preincubation of HepG2 cells with TNF-alpha followed by insulin significantly narrowed down insulin mediated exclusion of Foxa2 from the nucleus thereby substantially increasing its nuclear concentration and this possibly is responsible for the varied effects on gluconeogenesis and hepatic glucose output. TNF-alpha thus significantly abrogates insulin signaling in HepG2 cells leading to an increased nuclear presence of Foxa2 and subsequent elevated expression of gluconeogenic gene and glucose production. These results explain one of the mechanisms behind unrestrained hepatic glucose output that exaggerates an existing hyperglycemic status as observed in diabetic individuals.

299: Balancing the role of gene and environment: High-altitude adaptation and mal-adaptation

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High-altitude (HA) adaptation/mal-adaptation is a multifactor trait to which genetic and environmental factors contribute interactively. The traditional candidate gene approach for identifying molecular variants having functional role and associating with HA adaptation and disorders such as High-altitude pulmonary edema (HAPE) have achieved considerable success in elucidating individual's susceptibility. Identification of candidate genes still poses a great challenge. Since at HA, the adaptation/mal-adaptation is mainly characterized by induced pulmonary vasoconstriction, endothelial dysfunction and intra-vascular fluid retention, the genes involved in maintaining pulmonary vascular tone could be possible candidates. In a comparative study of highland (HL), lowland (LL) natives, and case-control i.e., HAPE patients-HAPE resistant sojourners, we investigated the polymorphisms insertion/deletion (I/D) (GenBank accession no X62855) of ACE, G-6A (rs5049); T174 M (rs4762) and M235T (rs699) of AGT; the G894T (rs1799983), 27 base pair 4b/4a (Ensembl Gene ID-ENSG00000164867), -922 A/G (rs1800779) and -786 T/C (rs3918161) of NOS3; the -344T/C (rs1799998), intron-2 conversion (Iw/Ic) (NCBI accession No. NW_924018) and Lys173Arg (rs4539) of CYP11B2 and (CT)n-(CA)n repeat (GenBank accession No. J05008), -3A/-4A (rs10478694), G2288T (rs2070699) and Lys198Asn (rs5370) of EDN1. Individual allele/genotype, combinations of genotypes, haplotypes, gene–gene interactions and relevant biomarkers were analyzed.

The allele/genotype distribution at the same locus varied significantly between different groups. The I, 894G and 4b, 2288G and longer repeats of -3A/-4A, and -344T allele frequencies were higher in HL than the LL ($p < 0.05$). Whereas, over-representation of D, 894T and 4a, 2288T and the Ic alleles was obtained in the HAPE-patients (p between 0.03 and 0.002) when compared against the HAPE-resistant controls. Combinations of genotypes analysis revealed over-representation of GGbb of NOS3, II/GG of ACE/END1 and the six/five major alleles of CYP11B2 in the controls and a reverse trend in the patients ($p < 0.05$). The higher ACE activity, endothelin and aldosterone levels and lower NO levels correlated with respective variants ($p < 0.0001$). A particular genetic setup of vascular homeostasis system in sojourners may help them cope with adverse HA environment and could ultimately be identified to predispose to adaptation and maladaptation.

300: Association of insertion deletion polymorphism in intron 16 of ACE gene with essential hypertension

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Several gene polymorphisms have been reported to be possible determinants of Hypertension of which the Angiotensin Converting Enzyme (ACE) that converts Angiotensin I to Angiotensin II, plays an important role in the elevation of blood pressure. ACE is a zinc metalloprotase containing two functional domains. ACE gene is located on chromosome 17q-23 and has 26 exons spanning 21 kb and is associated with vascular diseases. There is an Insertion/Deletion (I/D) of 287 bp in the intron-16 of the gene which shows polymorphism. The present study focuses on the risk conferred by I/D polymorphism for hypertension. The distribution of demographic parameters in 179 cases studied revealed a higher percentage of males (52.5%) when compared to females. The proportion of smokers was 20.67% and alcoholics 35.19%. There was a higher frequency of non-vegetarians among the hypertensives (81.56%). The frequency of familial history was higher in cases (55.86%) as compared to controls (40.8%). The age of onset of hypertension among patients varied from 35 to 60 years with a modal class of 40–50 years. The early age of onset of hypertension (<40 years) was recorded in 21.22% hypertensive patients. The frequency of obese subjects (BMI > 30) was twice (13.96%) in hypertensives as compared to controls (6.25%). The distribution of I/D genotypes revealed greater I/I genotype (37.03%) in cases ($n = 108$) when compared to controls (27.17%) ($n = 92$). Between the sexes, female hypertensives showed a higher percentage of I/I genotype (47.82%) when compared to male hypertensives (29.03%). Among the alcoholics a significant deviation ($\chi = 8.08$; $p < 0.017$) was observed within the cases. A significant deviation of I/I and D/D genotypes was observed in the females between the cases and controls ($\chi = 11.6$; $p < 0.002$). The odds ratio computed for genotypic combinations and alleles did not reveal any significant results. The study has to be substantiated with larger sample size.

301: Genetic predisposition to DNA repair and its effects on tumor response to radiation therapy in oral cancer

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Individuals differ in their susceptibility to disease. Some of these differences are attributed to the concept that heritable traits modify the effects of environmental exposures. Cancer of the oral cavity epitomizes this concept. Oral cancer, the eight most common malignancy and a major cause of cancer morbidity and mortality worldwide has become a significant social and economic burden in parts of South East Asia, particularly India. More than 80% of oral cancers are attributed to tobacco. However, only a fraction of smokers acquire oral cancer in their lifetime. One of the several cellular processes that could explain this inter-individual variation in risk is DNA Repair Capacity (DRC), which has been the focus of our study. There is a large subgroup with reduced DRC who are likely to be at

increased cancer risk, but are phenotypically normal. Our approach to risk assessment has been multitiered, beginning with a detailed epidemiological assessment in case-control studies, followed by the application of phenotypic and genotypic markers of genetic susceptibility. We first observed that SNPs in major DNA repair genes like XRCC1, XRCC3, ERCC1 and ERCC2 played a major role in predicting genetic susceptibility to oral cancer. We then looked into the actual extent of DNA damage, established by our in vitro studies using the Cytokinesis Block Micronucleus Assay [CBMN]. Higher spontaneous micronuclei levels observed in patients suggest a higher background level of genetic instability in the cancer patients. The next step was to look into DRC within oral cancer cases and control populations. This was done by the Host Cell Reactivation assay (HCR), which measures the expression level of UV damaged reporter genes. DRC% was correlated with SNPs in the DNA repair genes XRCC1, XRCC3, ERCC2 and ERCC1. Suboptimal DRC was observed in case of patients compared to control subjects which was associated with a two to threefold increased risk of oral cancer. It was seen that as repair capacity diminished, the probability of being a case increased. In addition to SNPs as cancer susceptibility markers, we also looked into their possible role as biomarkers of radiation treatment (RT) response. Patients with polymorphic variants in their genome responded better to RT as was evident from their complete response and no evidence of disease after a follow up period of 3 years. To the best of our knowledge this is the first study, which suggests a role for repair gene polymorphisms in influencing treatment protocols.

302: Molecular genetic analyses of tumours from colorectal cancer patients suggest existence of alternate tumourigenesis pathway(s) in young patients from India

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Colorectal cancer (CRC) is thought to be an age related disease, and usually occurs in older patients (>60 years). Familial syndromes resulting in an early occurrence of CRC account for a small proportion of CRC patients in the west and mainly include Familial Adenomatous Polyposis (FAP) and Hereditary Non Polyposis Colorectal Cancer (HNPCC). Deregulated WNT signaling and chromosomal instability have been reported in a majority ($\sim$70–85%) of CRC patients resulting from mutational inactivation of the adenomatous polyposis coli (APC) tumor suppressor gene. Another form of genetic instability, the Microsatellite Instability (MSI), has been shown to be responsible for approximately 15% of CRC cases. A rise in the incidence of CRC among the young has recently been reported in India. Unlike in the west these patients account for almost 1/3rd of the total CRC incidence (as per the local hospital registries) and often succumb to aggressive metastatic tumors. We have employed a multi-pronged strategy to study CRC occurring in young patients including (a) identification of status of WNT signaling through immunohistochemistry-based determination of intra cellular localization of β-catenin, (b) screening for mutations in the APC tumor suppressor gene, (c) screening for MSI, (d) identification of copy number alteration through microarray based Comparative Genomic Hybridization and (e) gene expression profiling using microarray and real time RT-PCR. Our results reveal that a significantly lower proportion (<40%) of tumors from young patients exhibit an active WNT signaling pathway, as opposed to tumors from older patients where the proportion is >75%, a statistically significant difference ($p < 0.007$, Fishers exact test). In addition, we identified several novel mutations in the APC gene from WNT + tumors. We could not detect any other significant difference between the two classes of patients with respect to microsatellite instability, tumor grade and stage, tumor location, gender, life style, etc. Our results therefore suggest the existence of alternate tumorigenesis pathway(s) in young sporadic CRC patients in India. We have initiated array-based determination of copy number alteration and transcript profiling to determine molecular signatures that govern rapid progression of WNT-tumors. Initial studies have revealed a novel amplicon at 7q22.3 in a WNT-tumor. The present study is expected to yield valuable insights into the molecular basis for young sporadic CRC.

303: Association of FCRL3 polymorphisms with juvenile rheumatoid arthritis in Mexican population

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Introduction: Juvenile rheumatoid arthritis (JRA) represents the most common rheumatic disease of childhood. Recently, Kochi, et al., reported four single nucleotide polymorphisms (FCRL3_3-6) in the FCRL3 gene strongly associated with susceptibility to several autoimmune diseases, including arthritis, in Japanese population. Furthermore, they observed that the allele C at the position-169C/T (FCRL3_3) alters the binding affinity of nuclear factor-kappa B affecting the FCRL3 expression, both in vivo and in vitro. FCRL3 gene encodes a member of the Fc receptor like family involved mainly in triggering activation of adaptative and innate effector cells, phagocytosis, release of inflammation mediators, cellular cytotoxicity.
Objective: The aim of this study was to determine if polymorphisms in the FCRL3 gene are associated with arthritis rheumatoid juvenile in a sample of Mexican pediatric patients. Samples and methods. In a case-control study we enrolled 157 patients, which meet the ACR classification criteria for JRA and 350 unrelated healthy controls without family history of autoimmune diseases. Both, cases and controls were stratified by sex and ethnic origin. The SNPs were genotyped ussing TaqMan assay. The association test, Hardy–Weinberg equilibrium (HWE) and haplotypes were determined using EPIDAT, FINETTI and Haploview softwares, respectively.
Results: Genotype distributions in cases and controls were in HWE. The results showed strong evidence of association of three SNPs across

FCRL3 gene with protection to JRA in this set of patients: FCRL3_3 (OR 0.54, 95% CI 0.34–0.82, p = 0.004), FCRL3_5 (OR 0.53, 95% CI 0.34–0.89, p = 0.003) y FCRL3_6 (OR 0.51, 95% CI 0.33–0.78, p = 0.002). Furthermore, when the sample was stratified by gender, distribution of the FCRL3 alleles reveled an association with protection only to JRA males: FCRL3–3 (OR 0.53, 95% CI 0.34–0.82, p = 0.004), FCRL3–5 (OR 0.48, 95% CI 0.30–0.74, p = 0.001), FCRL3–6 (OR 0.46, 95% CI 0.30–0.72, p = 0.0006). The haplotype harboring the 4 risk alleles CGCA, was also associated with protection to JRA (OR 0.53, 95% CI 0.37–0.75, p = 0.0003). The three SNPs showing association were on linkage disequilibrium (LD) (r2 0.88). Conclusion: Interestingly, our results suggest that while in other populations FCRL3 is associated with susceptibility to arthris in adults, in Mexican population this gene confer protection to development JRA and it seems that it is in a gender dependent manner.

304: Development of a web-enabled system for patient data management and genetic analysis of diabetes mellitus (DM)

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Diabetes mellitus (DM) is a complex multi factorial metabolic disorder, which involves genetic, epigenetic and environmental factors. It is characterized by high blood sugar, beta cell dysfunction and insulin resistance. Type 2 DM is the commonest form of diabetes, which accounts for 90–95% of the diabetes population resulting from insulin resistance combined with relative insulin deficiency. Type 1 DM is an autoimmune disease, which accounts for 5–10% of diabetic cases. A diabetes patient needs to be monitored for a number of clinical parameters such as blood sugar, cholesterol, insulin, blood pressure; environmental parameters such as stress, diet, exercise etc. Thus, the treatment procedure generates large amount of data. Therefore, the availability of the complete patient record would be useful to different specialists in rendering accurate treatment to the patient. With a view to manage the voluminous data and to assist the health care provider we have developed a web based data management system. It is a searchable, client-server, relational database application, developed on the Windows™ platform using Oracle, Active Server Pages (ASP), Visual Basic Script (VB Script) and Java Script. This system, apart from assisting the medical practitioner(s), is useful for analyzing the stored data as it provides the facility of generating reports by querying the database using specific parameters. An interactive web interface allows users to query the database and generate reports. Thus, this system can be implemented by Pharmaceutical companies, R&D laboratories, geneticists, population researchers, epidemiologists and nutritionists for research purposes. As an application of this database for research, we have studied inheritance of Diabetes mellitus in 3,921 individuals from 300 families in Maharashtra, India from the point of view of family history of DM and disease occurrence as well as pattern of inheritance of diabetes with respect to maternal or paternal transmission, age of onset etc. Detailed analysis of associated diseases has also been carried out. We have used molecular genetic analysis to detect the polymorphisms in the HNF1 gene in this population and have found SNPs and missense mutations that correlate with the incidence of DM. This analysis thus provides a platform to systematically analyze the complex interactions of environmental factors through epigenome which may play a role in presentation of phenotype in this complex multifactorial disease.

305: Interaction and integration of genes involved in immune responses with special reference to HLA-DRB1, HLA-B, Cytokine genes, Vitamin D receptor and protein tyrosine phosphatase non-receptor 22 genes in type 1 diabetes

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Type 1 diabetes (T1D) is a complex, multifactorial autoimmune disorder where at least 20 genomic intervals have been implicated. However, since the disease is autoimmune in nature, we have studied the interaction of alleles of genes involved in antigen presentation like HLA-DRB1 and HLA-B with functional SNPs in pro and anti-inflammatory cytokines, Vitamin-D-Receptor (VDR) and Protein Tyrosine phosphatase non-receptor 22 (PTPN22). Although encoded on different chromosomes, the products of these genes may interact in integrated networks resulting in the manifestation of T1D. 235 T1D patients were studied for HLA-DRB1 and HLA-B alleles using Polymerase chain reaction (PCR) and hybridization with sequence specific oligonucleotide probes using Luminex platform. The Single nucleotide polymorphisms (SNP) for IFN-gamma (INFG A + 874T), TNF-alpha (TNF G-308A), IL6 (G-174C), IL10 (A-1082G, T-819C, C-592A), and TGF-beta1 (TGFB1 Tcdn10C, Gcdn25C) were studied using PCR with sequence specific primers. Four single nucleotide polymorphisms in the VDR gene (Fok1 site in Exon 2, Bsm1 and Apa 1 sites Intron 8 and Taq1 site in exon 9) were studied using PCR–Restriction Fragment Length Polymorphism (PCR–RFLP) and a functional SNP in the PTPN22 gene in the codon 620 at the nucleotide position 1858C > T was also studied using PCR–RFLP.

An in-depth analysis of the data shows that while predisposing HLA alleles, HLA-DRB1*0301, DRB1*0401 and DRB1*0405, may have a role in antigen presentation, products of cytokine genes whose amounts may be determined by SNPs in the cytokine genes, VDR gene and PTPN22 also have integrated functional roles in manifestation of the disease. VDR may have a role in the regulatory function of 1,25–(OH)2D3 since vitamin D3 is the ligand for VDR and Vitamin D3, in turn, has been shown to have an important immunomodulatory role. PTPN22 which codes for a protein called lymphoid tyrosine phosphatase (LYP) has been shown to be involved in preventing spontaneous T-cell activation by dephosphorylation and inactivating T-cell receptor associated kinases and their substrates. The SNP in the codon 620 of PTPN22 gene results in an arginine to tryptophan transition which may reduce the binding affinity of LYP to cytoplasmic tyrosine kinase (Csk), which regulates the T-cell receptor signaling. Data on interaction integration of these genes and the implications there off to give the final verdict of Type 1 diabetes will be presented.

306: Association between CLEC7A gene and pneumocystis pneumonia (PCP) susceptibility among HIV-infected individuals

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Pneumocystis pneumonia (PCP) is a fungal lung infection caused by *Pneumocystis jirovecii* that remains a leading cause of morbidity and mortality among HIV-infected individuals. PCP is extremely rare in people with normal immune function but common among immunocompromised individuals. Although the innate and acquired immune mechanisms against *P. jirovecii* are not fully understood, initiation of an immune response is believed to be triggered by host recognition of fungal beta-glucan cell wall components. Earlier studies have shown that recognition of beta-glucan is partly mediated by the pattern-recognition receptor CLEC7A (C-type lectin domain family 7, member A; also known as Dectin-1). In the current study we aimed to assess whether polymorphisms in the human CLEC7A gene affect susceptibility to PCP among 263 HIV-infected UK Caucasian individuals. Nine haplotype-tagging SNPs spanning a 15 kb region across CLEC7A on chromosome 12 were genotyped using Sequenom's hME chemistry. As CD4 + T cell deficiency clearly correlates with susceptibility to PCP, CD4 + cell count was included as a covariate in the logistic regression model (SPSS 16.0 for Windows). One SNP in the first intron (allelic $p = 0.037$; OR 2.74, 95% CI 1.06–7.06) and the adjacent SNP in the promoter region of the gene (allelic $p = 0.0038$; OR 4.01, 95% CI 1.57–10.28) showed apparent association with PCP-susceptibility in the setting of HIV infection. Based on LD structure, these nine genotyped SNPs can be divided in two distinct haplotype blocks, with the two associating SNPs located in the second block. Haplotype analysis of three SNPs in this second block revealed one risk haplotype that can be tagged by the associating promoter SNP ($p = 0.0038$; OR 4.59, 95% CI 1.63–12.88) and one protective haplotype ($p = 0.0012$; OR 0.42, 95% CI 0.25–0.71) (haplotypes computed with a SNPHAP software). Since the sample size is too small to make firm conclusions, a replication study of 319 Danish HIV-infected individuals is currently ongoing. Studies of host genetic factors affecting susceptibility to PCP are scarce; the current study suggests that host genetic factors may play an important role in susceptibility to PCP among immunocompromised individuals.

307: Glutathione-*S*-Transferase P1 (GSTP1) polymorphism and breast, colorectal and oral cavity cancer risk among Filipinos

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Cancer is a multi-factorial disease that may occur as a result of the interaction of genetics and the environment. Genetic factors by themselves are thought to explain only about 5% of all cancers while the remainder can be attributed to environmental factors that act in conjunction with both genetic and acquired susceptibility. Numerous population based studies have honed in on glutathione-*S*-transferase genes as candidate cancer susceptibility genes because of their involvement in the conjugation of glutathione to a multitude of carcinogens and their reactive intermediates thereby facilitating detoxification and subsequent excretion. This particular study employed only Filipino subjects and explored the association between the c.313A > G (p.I105 V) GSTP1 polymorphism in exon 5, and incidence risk for 3 cancer sites: oral cavity (195 cases and 315 controls), colon and rectum (216 cases and 268 controls), and breast (327 cases and 223 controls). Statistical analysis relating cancer risk to various epidemiologic risk factors such as diet, chemical exposure and medical history was also performed. Genotyping was done using PCR–RFLP, and results were verified via direct sequencing. For breast and colorectal cancer, matched univariate analysis revealed that none of the GSTP1 genotypes yielded a statistically significant result; for oral cavity cancer, the homozygous variant genotype (Val/Val at amino acid pos. 105) was shown to significantly increase individual risk for the disease (OR = 2.04, 95% CI: 1.01–4.12). These results show that GSTP1 genotype has no effect on breast nor colorectal cancer risk among Filipinos, while the homozygous c.313A > G genotype is a significant risk factor for oral cavity cancer.

308: Enhancement of risk of oral leukoplakia and cancer by combinations of polymorphisms at NAT1, NAT2 and XRCC1

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Introduction: The *N*-acetyl transferases (NATs) are important enzymes that could acetylate N- or C-positions of tobacco carcinogens for detoxification or activation. Improper activities of these enzymes might lead to the formation of DNA adducts that need correction by DNA repair enzymes otherwise DNA adducts may progress to mutation and finally tumor progression.
Methods: Here, genotypes at four SNPs on *NAT1,* five SNPs on *NAT2* and three SNPs on *XRCC1* were determined by PCR–RFLP and Taqman methods in 389 controls, 224 leukoplakia and 310 cancer patients. Genotypes at these loci were also expressed as haplotypes and diplotypes by a maximum-likelihood method using the expectation maximization algorithm. Individuals can be assigned as NAT1 rapid, NAT1 normal, NAT2 slow, NAT2 intermediate and NAT2 rapid acetylators depending on the corresponding diplotypes present in an individual. Genotype, haplotype and diplotype data at individual locus and in combinations were analyzed to estimate the risk of oral leukoplakia and cancer.
Results: Genotypes and diplotypes at *NAT1* and *NAT2* could not modify the risk of diseases in this population. Genotypes at *XRCC1* could not modify the risk of diseases but variant haplotypes could increase the risk of leukoplakia marginally. The *XRCC1* variant haplotypes could also increase the risk of both cancer and leukoplakia in mixed tobacco habitués only. Combining the *NAT1* or *NAT2* and *XRCC1* data in mixed tobacco habitués, it was observed that *XRCC1* variant haplotypes enhanced the risk of leukoplakia and cancer with increase in risk level (OR = 3.8, 95% CI = 1.7–8.5 and OR = 2.9, 95% CI = 1.4–6.2, respectively) in *NAT1* rapid and (OR = 3.8, 95% CI = 1.9–7.7 and OR = 2.8, 95% CI = 1.4–5.5, respectively) in *NAT2* slow acetylators compared to the risks attributed by *XRCC1* variant haplotypes alone. Interestingly, combination of *NAT1* rapid, *NAT2* slow and *XRCC1* variant haplotypes increased the risk of leukoplakia and cancer with enhancement of more risk (OR = 5.4, 95% CI = 1.8–16.5 and OR = 3.7, 95% CI = 1.3–10.6, respectively).
Conclusion: None of the genotypes at these loci could modify the risk of diseases independently but those at two or more loci, in combination, could increase the risk significantly in subsets of patients.

309: Polymorphisms in the IL6 gene in Asian Indian families with premature coronary artery disease: The Indian Atherosclerosis research study

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Inflammation plays a major role in coronary artery disease (CAD). Interleukin-6 is an important inflammatory marker and a risk factor of CAD. The present study was undertaken to investigate the association of the interleukin-6 gene (IL6) polymorphisms with premature CAD and their effect on the expression of acute-phase proteins in Asian Indian families. One hundred and ninety affected sib pairs (ASPs) were genotyped for three tag single nucleotide polymorphisms (SNPs) of the IL6 gene by TaqMan allelic discrimination assays. We observed suggestive linkage for one SNP (rs2066992) in a subset of 62 ASPs with an age at onset less than 45 years (LOD score = 1.114, $p = 0.011$ in linkage analysis; pi = 0.008 in identity by descent). Quantitative trait loci analysis indicated linkage of the genotypes with plasma levels of high sensitivity C-reactive protein, hsCRP (LOD score = 1.06, $p = 0.014$). Sequencing of the promoter region and haplotype analysis was performed in 46 probands and 40 controls. Five out of eight previously reported promoter SNPs were found to be polymorphic in our cohort. Two novel sequence variants were found. A promoter haplotype (GGAAG) was found to be associated with premature CAD with an odds ratio of 3.676 ($p = 0.0017$, 95% CI: 1.68–8.045). The plasma levels of both hsCRP and fibrinogen exhibited significant association with these promoter SNP genotypes ($p < 0.001$). In conclusion, IL6 gene polymorphisms appear to be important genetic factors in premature CAD, and in the regulation of key atherogenic markers in Asian Indian families.

310: Metabolic enzyme gene variants and oral cavity cancer among Filipinos

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Oral cavity cancer is a leading cause of cancer among Filipinos, with a 34% observed 5-year survival rate. Previous studies in other populations have looked into the genetic risk for cancer which is conferred by variants in xenobiotic-metabolizing enzyme genes. In this study, DNA from 195 cases and 315 controls were genotyped via PCR–RFLP and direct sequencing. After univariate analysis using age-matched logistic regression, it was shown that homozygosity for the c.313A > G GSTP1 allele increased risk for oral cavity cancer (OR 2.04; 95% CI: 1.01, 4.12), while homozygosity for the 3′UTR polymorphism of CYP1A1 has a protective effect (OR 0.52; 95% CI: 0.29, 0.92). The effect modification of various environmental factors, such as smoking, alcohol and salty food intake and UV exposure were also studied. These results may be helpful in elucidating the gene–gene and gene–environment interactions which predispose to oral cavity cancer among Filipinos.

311: Novel genetic variations in TNNT2 gene are associated with hypertrophic and dilated cardiomyopathies of Indian origin

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Cardiomyopathies are diseases of heart muscle that may result from a diverse array of conditions that damage the heart and impair myocardial function. Due to the genetic heterogeneity and the variable clinical expressivity of these diseases, the relations between genotype and phenotype remain complex. Since Indian populations are prone to high degree of cardiac disorders, due to their unique population structures and exposure to different life style, this study was aimed to screen for mutations in cardiac trophonin T2 (TNNT2) gene in Indian patients with hypertropic (HCM) and dilated cardiomyopathy (DCM). We have analysed all the exons, including the exon-intron boundaries of the above genes in a total of 384 patients, (including 200 HCM and 184 DCM patients) along with 110 ethnically matched controls. Mutation analysis revealed a total of five exonic mutations, of which three of them were novel and non-synonymous mutations changing the amino acids. Of the three mutations, one (A27 V) was observed in a HCM patient and the remaining two (R141Q, R144W) were observed in DCM patients. One synonomous mutation found only in dilated cardiomyopathy. Interestingly, one splice acceptor site mutation was observed in the exon 12 of a dilated cardiomyopathy patient. Seven intronic mutations were observed in introns 5, 6, 11, 12, 15 and 16. Further, 5 bp deletion/deletion polymorphism in intron 3 and its association with predisposition to HCM was also studied. Our analysis revealed that the Indian populations have significant number of novel mutations, which could be responsible for the additional genetic causes. A details data with functional significance would be presented during the conference.

312: Genetic studies on the APOA1-APOC3-APOA5 gene cluster in Asian Indians with premature Coronary Artery Disease

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The APOA1-APOC3-APOA5 (Apo11q) gene cluster, located in the 11q23-24 chromosomal region, plays an important role in lipid regulation. Given that Asian Indians have a tendency towards exhibiting abnormal lipid levels and carry a high risk of coronary artery disease (CAD), the present study aimed to elucidate the relationship of four single nucleotide polymorphisms (SNPs) in the Apo11q cluster ($-75G > A$ [rs1799837] and $+83C > T$ [rs5069] SNPs in the APOA1 gene, the $3238C > G$ [rs5128] SNP [Sac1] in the APOC3 gene and the S19 W [rs3135506] variant in the APOA5 gene) to circulating levels of plasma lipids and CAD in 190 affected sibling pairs (ASPs) belonging to Asian Indian families with strong history of premature CAD. Genotyping and lipid assays were carried out using standard protocols. Plasma lipids, namely Total cholesterol (TC), Triglycerides (TG), High density lipoprotein-cholesterol (HDL-c), showed strong heritability ($h2 > 48–70\%$; $p < 0.0001$). A subset of 77 ASPs showed significant linkage to CAD trait by multi-point analysis (LOD score 7.42, $p < 0.001$) and to the Sac1 (LOD score 4.49) and the $-75G > A$ (LOD score 2.77) SNPs by single-point analysis ($p < 0.001$). There was significant proportion of mean allele sharing (pi) ($p < 0.001$), identity by descent, for the Sac1 (pi 0.59), the $-75G > A$ (pi 0.56) and the $+83C > T$ (pi 0.52) SNPs, respectively. Quantitative trait loci analysis demonstrated suggestive evidence of linkage of the Sac1 SNP to TC, HDL-c and Apolipoprotein B (LOD score 1.42, 1.72, 1.19, respectively; $p < 0.01$). The Sac1 and the $-75G > A$ SNPs along with hypertension showed maximized correlations with TC, TG and Apolipoprotein B levels. In conclusion, the Sac1 SNP in the APOC3 gene is an important genetic variant for premature CAD through its association with established risk factors such as dyslipidemia among Asian Indians.

313: Role of OPRM1 gene in T2DM susceptibility and its prevalence in North-West part of Rajasthan

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Prevalence of Type 2 Diabetes Mellitus (T2DM) varies from <2% in rural Kashmir to >20% in urban areas of Hyderabad as reported by different studies in India. International studies also report a wide variation in T2DM prevalence. Genetic differences as well as dietary factors have been implicated to explain these regional differences. In India the prevalence of diabetes in the Riaca Community of North-Western Rajasthan is extremely low, <0.5%. Agrawal et al. (2004) conducted a cross-sectional survey of representative Raica and Non-Raica community subjects and reported that both fasting as well as post-glucose load, the glucose levels were significantly lower in Raica community as compared to the Non-Raica individuals in the same region. Agonists of mu-opioid receptor (OPRM1) affect glucose-induced insulin release as seen in OPRM1 knockout mice which have a more rapid induction of insulin resistance than their wild-type counterpart. OPRM1 thus serves as an attractive positional candidate gene for T2DM susceptibility. Gallagher et al. (2006) indeed detected linkage of T2DM to 6q24-q27 using a genome-wide scan in African Americans (AA) families and OPRM1 gene is located within the 6q24-q27 region. These associations suggested that the OPRM1 gene may play a role in T2DM susceptibility in AA. Taking cue from these reports the present study aims to delineate the frequency of C17T (Ala6Val) and A118G (Asn40Asp) SNPs in exon I of OPRM1 gene in subjects of Raica ($n = 58$) and Non-raica ($n = 40$) community and study their association with the incidence of T2DM in diabetic subjects ($n = 39$) from north-west Rajasthan. C17T (rs 1799972) and A118G (rs 1799971) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. A significant association was observed between the C17T (Ala6Val) allele and no association was seen with A118G (Asn40Asp) polymorphism and T2DM. The frequency of 17T allele was significantly higher in Raica (0.48), in comparison to Non-Raica (0.30) ($p = 0.0159$) and non-raica diabetic (0.36) subjects population whereas no difference was observed in the frequency of 118G allele. The raica community members did not have any cases of T2DM for comparison. The population of northwestern Rajasthan in general seems to have a higher prevalence of 17T allele as compared to other regional populations from India. In the North Indians C17T reveals a frequency of 0.11 whereas a frequency of 0.45 is observed in individuals of Rajasthan origin.

314: MTHFR gene polymorphisms among Aggarwals: A community specific case control study for CAD

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Coronary Artery Disease (CAD) is the leading cause of death in world which is belived to be caused by many genetic and environmental factors. MTHFR gene is one of the 400 reported candidate gene for CAD. MTHFR enzyme converts 5, 10 Methylenetetrahydro folate to 5-Methylenetetrahydro folate, the failure of which causes increased levels of Homocystein in blood. The present study attempts to assess the role of two polymorphisms 677T-C and 1298A-C in CAD. 100 CAD patients and 100 age, sex matched controls are screened for the mutation among Aggarwals of Delhi. The mutated allele T (28%) and C (22%) of C677T and A1298C polymorphism, respectively, are found to be in higher frequency among cases. Significant difference between cases and controls with an odds ratio i.e., 2.29 (95% CI = 1.22–4.28) and 2.03 (95% CI = 1.047–3.94) for C677T and A1298C, respectively, indicates that the two mutation are risk factors for CAD, specifically among Aggarwal community.

315: Role of DNA Repair gene polymorphisms in gallbladder cancer

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Introduction: Gallbladder cancer (GBC) is the most common biliary tract cancer with highest incidence rate in North India. Carcinogenesis has been linked with increased DNA damage by endogenous or

exogenous agents like reactive oxygen species (ROS), free radicals and peroxides. Cells overcome these damages by specialized DNA repair pathways one of which is base excision repair (BER) pathway. Genetic polymorphisms in OGG1 and XRCC1, two important enzymes participating in BER pathway may be involved in influencing inter-individual variations in DNA repair capacity, which may be associated with susceptibility to GBC.
Objective: This study was aimed to examine the role of OGG1 Ser326Cys (C > G, rs1052133), XRCC1 Arg399Gln (G > A, rs1799 782) and XRCC1 Arg194Trp (C > T, rs25487) polymorphisms in susceptibility to GBC.
Methodology: A case-control study (173 GBC patients and 204 controls) was used to test the association between the three polymorphisms and risk of GBC. Genotyping for all three polymorphisms was done by PCR–RFLP method.
Results: All the studied polymorphisms were consistent with Hardy–Weinberg equilibrium in controls. OGG1 Cys/Cys genotype frequency was significantly higher in GBC patients [p = 0.025; Odds Ratio (OR) = 2.93; 95% Confidence Interval (CI) = 1.14–7.51]. The increased risk was more pronounced in GBC patients with gallstones (p = 0.001; OR = 5.50; 95% CI = 1.99–15.16), females (p = 0.029; OR = 5.92; 95% CI = 1.20–29.13) and late onset of disease (>50 years age) (p = 0.010; OR = 4.72; 95% CI = 1.43–15.53). In XRCC1 Arg399Gln polymorphism, Gln/Gln and Arg/Gln genotypes conferred low risk for the disease. (p = 0.039 and 0.003; OR = 0.68 and 0.37, respectively). However, XRCC1 Arg194Trp polymorphism was not associated with GBC risk. Also, carriers of Arg-Gln haplotype of XRCC1 were at significantly low risk for GBC (p = 0.002; OR = 0.59, 95% CI = 0.42–0.82). The interaction of genotypes and tobacco usage did not modulate the risk for GBC.
Conclusion: Results suggest that Cys/Cys genotype of OGG1 Ser326-Cys polymorphism is associated with GBC susceptibility. However, further studies are needed to strengthen its role in GBC pathogenesis.
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316: Dilated Cardiomyopathy caused by a mutation in Phospholamban

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Dilated cardiomyopathy (DCM) is a disorder of the cardiac muscle. Dilated cardiomyopathy inherits in an autosomal dominant mode. The estimated prevalence is 1:500 in the general population and has worldwide distribution among all ethnic groups. Patients with DCM exhibit clinical manifestations that vary from an asymptomatic condition to that of severe heart failure that leads to sudden cardiac death. Here we report that an inherited human dilated cardiomyopathy with refractory congestive heart failure is caused by a dominant mutation in phospholamban (PLN), a transmembrane phosphoprotein that inhibits the cardiac sarcoplasmic reticular Ca^{2+}-adenosine triphosphatase (SERCA2a) pump. It is also clear that the variations do not fully explain the degree of variability in the phenotypic expression. The patients were evaluated by clinical history, physical examination, electrocardiogram and echocardiography. All PCR products were analyzed for mutation by sequencing. It is expected that this finding would provide direct evidence for the hypothesis that there is deleterious to the heart. Thus, we sought to determine the association between the phenotype and genotype in an Indian family. These results indicate that myocellular calcium dysregulation can initiate human heart failure—a finding that may lead to therapeutic opportunities.

317: APOBEC3 Insertion/Deletion polymorphism among patients infected with hepatitis B virus

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The replication of Hepatitis B virus (HBV) proceeds via an obligatory reverse transcription step in the viral capsid. cDNA is potentially vulnerable to editing by cytidine deaminases of the APOBEC3 family. G to A hypermutation of HBV and retroviruses appears as a result of deamination activities of host APOBEC proteins and is thought to play a role in innate antiviral immunity. Alpha and gamma interferons have been reported to upregulate the transcription of APOBEC3G, which is known to reduce the replication of HBV. Several recent studies have brought increased attention to classes of genomic variation such as deletions, inversions, and copy-number polymorphisms. It is thought that these variations contribute substantially to inter-individual genotypic, and perhaps phenotypic, variation, but the structure and population characteristics of these variants remain largely unexplored. A deletion in the APOBEC3 gene cluster was recently identified that spans ~ 350 bp. Individuals possessing this structural variant would lack at least one copy of the unique coding portion of APOBEC3B. In this study, we report a deletion polymorphism in normal and HBV positive patients which results in APOBEC3B deletion. In 96 normal individuals, the deletion frequency was 1.39% revealing that there is low preference in favor of deletion in this population as compared to other populations (Kidd et. Al., unpublished). To understand the influence of this deletion on HBV infection, we investigated the insertion/deletion polymorphism of APOBEC3 gene in 75 patients with chronic hepatitis B (CHB) and 79 non-CHB patients by a genotyping PCR (Kidd et al., unpublished). The frequency of homozygous deletion was 1.33 and 1.26%, respectively, and that of heterozygous insertion/deletion was 24 and 22.78%, respectively, for CHB and non-CHB patients. Thus, the deletion frequency within the APOBEC3 gene in HBV positive patients is similar to that in normal individuals. It has been shown that exposed ssDNA regions in the gene undergoing transcription are the targets of APOBEC3 deaminases causing mutation in the pathogen genome. Thus, the presence of APOBEC3B gene is more likely to be in an environment where there is high viral threat, similar to our observations. However, a high insertion frequency indicates the presence of APOBEC3B in most of the patients, contrary to our expectation. The answer may lie in the variable expression levels of APOBEC3 in the liver cells, this, however, warrants further investigations.

318: Genetic variants of FOXA2: Risk of type 2 diabetes and effect on metabolic traits in North Indians

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Normal levels of blood glucose are maintained through integrated mechanisms of glucose sensing, insulin production and glucose utilization. Hence, pancreatic β-cells occupy the central role in

maintaining glucose homeostasis and defects in its development and function can result in imbalances in glucose metabolism and diabetes. Hence, FOXA2 being an upstream activator of β-cell transcription factor network regulating the development and function of adult β-cells might be a key player in the manifestation of type 2 diabetes. Therefore, here we examined the association of genetic variants of FOXA2 with type 2 diabetes and related phenotypes in North India. We performed case-control analysis of FOXA2 SNPs (rs1212275, rs1055080, rs6048205) and (TCC)n repeat polymorphism in 1,656 North Indian participants of Indo-European ethnicity including 1,031 patients with type 2 diabetes and 625 control subjects. SNPs rs1212275 and rs6048205 were found to be uncommon (MAF $< 5\%$) with similar distribution among patients and controls. We found strong association of (TCC)n common allele A5 and type 2 diabetes with A5 homozygotes having odds ratio of 1.66 (95% CI 1.36–2.04, $p = 5.9 \times 10^{-7}$). Obese individuals with A5A5 genotype had enhanced risk of type 2 diabetes when segregated from normal-weight subjects [OR = 1.92 (95% CI 1.47–2.51), $p = 1.6 \times 10^{-6}$]. A5 was also nominally associated with higher fasting glucose ($p = 0.02$) and lower fasting insulin ($p = 0.0028$) and C-peptide ($p = 0.036$) levels among controls. At rs1055080 locus, GG was found to provide reduced risk among normal-weight subjects [OR = 0.59 (95% CI 0.40–0.88), $p = 0.011$]. Combination of protective GG and non-risk genotypes of (TCC)n showed reduced risk of type 2 diabetes both among normal-weight [OR = 0.43 (95% CI 0.29–0.65), $p = 1.2 \times 10^{-6}$] and obese individuals [0.47 (95% CI 0.34–0.64), $p = 4.3 \times 10^{-5}$]. For the first time we demonstrate that FOXA2 variants may influence the risk of type 2 diabetes and affect the metabolic traits in North India with (TCC)n locus and rs1055080 having opposing effects.

319: Risk conferred by Leptin (LEP) and Leptin receptor (LEPR) gene polymorphisms to essential hypertension irrespective of obesity

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In the present study 280 hypertensives and 200 normotensives were studied to evaluate the risk of leptin (LEP) and leptin receptor (LEPR) polymorphisms (a terta nucleotide polymorphism) for developing essential hypertension. LEP and LEPR genotyping was done by PCR amplification and separation of the short or class I (<160 bps) and longer or class II (>160 bps) alleles for LEP and short(S, <158 bps) and longer (L, >158 bps) alleles for LEPR genes using 9% acrylamide gels.

There was higher prevalence of males (54.6%) among hypertensives with 66.3% of the cases having positive family history which was twice that is found in controls. Age at onset of hypertension varied from 35 to 60 years with modal frequency lying between 40 and 55 years. Biochemical analysis showed an increase in the mean levels of triglycerides and decrease in HDL in hypertensives as compared to controls. Distribution of genotypes of LEP gene differed significantly (χ^2 −12.72, $p = 0.001$) between patients and controls (22.9% of I/I, 25.4% of I/II and 51.8% of II/II in hypertensives and 15.0% of I/I; 40.0% of I/II and 45.0% of II/II in controls). This suggests protection for heterozygotes against hypertension and high risk was for both the homozygotes. Stratification of the data revealed significant variation in the distribution of LEP genotypes in males (χ^2 −6.87, $p = 0.03$) cases with positive family history (χ^2 −11.5, $p = 0.003$), low BMI (χ^2 −10.7, $p = 0.005$) and those with the habit of smoking (χ^2 −6.25, $p = 0.044$) and alcohol consumption (χ^2 − 11.5, $p = 0.004$). Lipid profiles though were elevated in hypertensives did not differ significantly from controls and also between different genotypes within the patient and control groups. Distribution of LEPR genotype did not vary significantly though there was elevation of homozygosity for longer fragments (L/L) in patients as compared to controls. When interaction between the genotypes of LEP and LEPR were tested, risk for hypertension was found to be higher in individuals with LEP I/I and LEPR L/L combination of genotypes as compared to controls. Leptin gene which is strongly associated with obesity has not shown any difference between obese and non-obese hypertensive patient suggesting that the association of LEP polymorphisms found in present hypertensives cases is independent of obesity.

320: Genetic Predisposition of MTHFR Polymorphism in Coronary Artery Disease (CAD): SGPGI Experience

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Obstructive coronary artery disease (CAD) is characterized by the deposition of atherosclerotic plaque on the coronary artery wall. Its manifestations depend on interactions between environmental and genetic risk factors. The Atherogenic profile of plasma lipoproteins is characterized by elevated concentration of plasma triglycerides (TG) and by a predominance of small dense LDL and low HDL cholesterol. Small HDL particles and a lack of larger HDL particles also contribute to the plasma atherogenic profile. Aim of the study was to analyze the frequency of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in patients with CAD and its association with atherosclerotic index of plasma (AIP) levels. Risk factors for CAD were also evaluated. A Prospective case control study has performed. Two hundred and forty-nine angiographically proven CAD patients and two hundred twenty three age sex matched healthy individuals as control were studied. All individual filled a clinical performa to analyze risk factors for CAD. MTHFR polymorphism was investigated by restriction fragment length analysis and correlated with the number of affected arteries and degree of arterial obstruction determined by coronary angiography, and biochemical investigations in all individuals. AIP were calculated by calculating log TG/HDL. The T allele frequency was found insignificantly associated with coronary artery disease ($p = 0.078$, OR 1.51), However, in terms of risk factor associated with CAD the T allele frequency has found significantly associated with hypertension ($p = 0.031$, OR 2.12), Diabetes ($p = 0.032$, OR 2.04) and Smoking ($p = 0.019$, OR 2.38) and insignificantly associated with positive family history ($p = 0.70$). While Atherosclerotic index of plasma was found to be significantly ($p = 0.04$) associated with C677T polymorphism. The present study indicates that CT + TT genotype in patient group is insignificant associated with number of obstructed vessels ($p = 0.69$).

321: Molecular markers in idiopathic pulmonary arterial hypertension

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Idiopathic Pulmonary Arterial Hypertension is a disease characterized by sustained elevation of mPAP >25 mmHg at rest or >30 mmHg during exercise, without an underlying cause. There is progressive narrowing of the Pulmonary artery, that impedes the blood flow from the right ventricle to the lungs, eventually leading to right ventricular failure. IPAH is characterized by vasoconstriction of the small pulmonary arteries, proliferation in all layers of the vessel wall, thrombosis-in situ, and inflammation. The disease is rare with estimated incidence being 1–2 cases per million in the general population. The etiology of the disease still remains obscure. In the present study genotyping of SLC6A4, SERPINE1, IL6 and IL1B genes and SSCP analysis of the EDN1 (endothelin 1) and BMPR2 (Bone Morphogenetic Protein Receptor II) genes was carried out for 54 IPAH cases and 100 controls to identify possible genetic/molecular risk markers. The BMPR2 gene is the identified candidate gene for IPAH. We identified only 1 known mutation in exon 3 in 5 patients but in Indian population it appears to have low frequency (9.25%), clearly indicating genetic heterogeneity of the disease. Screening of EDN1 gene revealed a known polymorphism-Ins A at +138 nucleotide position from the transcription initiation site in 8 IPAH patients and 3 controls. Genotypying of SLC6A4 promoter showed an increased risk of IPAH with LL genotype when compared to SS genotype (OR-3.106, CI-1.203, 8.095). OR of LS versus SS genotype was also highly significant (OR-5.538, CI-2.185, 14.274). This data further supports the implicated role of L allele in pathogenesis of IPAH. Significant association of Hd2/Hd2 genotype of SERPINE1 was observed with IPAH as compared to Hd1/Hd1 and Hd1/Hd2 genotypes (OR-3.12, CI-1.08, 9.17). PAI-1 determines in part fibrinolysis activity and modulates the progression of thrombosis. Thus, impaired fibrinolytic activity could play an integral role in the development of thrombosis as seen in the disease. The odds test of association was also found to be significant for CC genotype of (-511) IL1B gene with IPAH cases when compared to TT genotype (OR-2.59, CI-1.09, 6.17). IL-1 β is a proinflammatory cytokine that may play significant role in proliferation of endothelial and smooth muscle cells. The study has brought out the interactive role of these markers and identification of susceptibility alleles influencing disease progression with poor prognosis.

322: Genome wide study of tuberculosis susceptibility in West Africans reveal novel associations

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Tuberculosis continues to cause substantial morbidity and mortality throughput the world, especially in developing countries where infrastructure and access to treatment is limited. Host genetics play an important role in tuberculosis disease as demonstrated by twin studies. Although many tuberculosis candidate gene studies have been published many fail to replicate. We performed a genome wide association study of tuberculosis susceptibility in a Gambian population to determine genetic loci associated with tuberculosis disease process. DNA was collected and genotyped for 1,498 pulmonary tuberculosis cases and 1,496 population controls from The Gambia. In total 429,403 SNPs were analysed to find 38 independent loci associated with tuberculosis disease with P less than 10^{-4} and seven loci with P less than 10^{-5}. The analysis revealed that several of the associated genes are all related to NCAM1, suggesting a role of this gene family in the development of pulmonary tuberculosis. Replication studies are currently underway to attempt to confirm these findings. Functional analysis of two of the loci associated with tuberculosis has revealed differential TNF production based upon genotype, suggesting a mechanism of protection against tuberculosis.

323: SNPs in *FTO* and *GNB3* are associated to obesity in Mexican Mestizos

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Introduction: Morbid obesity is considered a global pandemia. Approximately two thirds of the Mexican population are overweight with a Body Mass Index (BMI) > = 25 kg/m2, and close to 25% are clinically obese. Previously published studies have suggested that Hispanic Americans have a higher risk for the disease. As in other Hispanic populations, most Mexicans are Mestizos, individuals resulting from admixture of any of 65 ethnic groups, with Spaniards, an in a lesser extent Africans. In order to increase our understanding of the genetic basis of obesity in Mexicans, we aimed to analyze a number of genetic polymorphisms already reported to be associated to obesity in other populations.

Materials and Methods: We genotyped a set of 23 SNPs in a case-control design in Mexicans. The selection of the SNP set was based on their reported statistical significant association to obesity, replication and biological relevance. Cases and controls were selected according to their BMI ($\geq$30 and $\leq$27 kg/m^2, respectively). Ninety-two controls and 138 cases were included (mean BMI $\pm$ SD in cases = 26.7 $\pm$ 4.3 kg/m^2 and in controls = 46.8 $\pm$ 10 kg/m^2, $p < 0.0001$). About 50% of cases reported to have a first-degree obese relative and/or to have obesity since childhood. We genotyped 23 SNPs in 13 genes: *ADIPOQ* (rs1501299, rs2241766, rs822396), *LEPR* (rs1137100, rs1137101, rs8179183, rs13306526), *LEP* (rs17151919), *GNB3* (rs5443), *MC3R* (C_27859133), *MC4R* (rs13447335, rs13447324, rs13447332), *NR3C1* (rs6195, rs33391, 6192), *AGRP* (rs5030980), *ADRB3* (rs4994), *ADRB2* (rs1042714), *PPARG* (rs1801282), *POMC* (POMC87, rs1042571) and POMC158, rs2071345) and *FTO* (rs8050136). To test for association we used traditional Chi square analysis and Cochran–Armitage's trend test.

Results: Our results showed evidence of association for rs5443 in *GNB3* (OR = 1.7 95% CI = [1.2–2.6], p = 0.007) and for rs8050136 in *FTO* (OR = 2.1 95% CI = [1.3–3.4], p = 0.002). In addition, we observed that females were more obese than males (p = 0.0001), and both, fat percentage and waist & hip ratio were significantly different among the two groups ($p < 0.0001$).

Conclusion: We found evidence that variants in *GNB3* and *FTO* are moderately associated to obesity in Mexican Mestizos. In order to increase statistical power in our study we are increasing sample size and further analyzing population stratification in our sample.

324: Genetic studies in type 2 diabetes in south Indian population

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Type 2 diabetes results from the interaction of environmental factors with genetic variants which confers susceptibility to the disease. Recently, a systematic search for these variants using genome wide approach was made possible the genotyping of thousands of polymorphisms and identification of new risk alleles for T2D. Some of these have been replicated in multiple populations and provide novel insights into the etiology of the disease. Evidence for increasing prevalence of diabetes in India arises from the recent population based studies conducted by us, the Chennai Urban Rural Epidemiology Study(CURES), which revealed the present prevalence of diabetes in urban India as 72% higher than that reported in 1989. Indians with diabetes also have peculiar characteristics called Asian-Indian phenotype. This includes low body mass index threshold for diabetes, occurrence of the disease 2–3 decades earlier and heritability factors being stronger in Indians compared to Europeans pointing to the role of possible ethnic variation in genetic susceptibility. Large population based genetic studies carried out by us on some of the candidate genes or risk alleles, indicated interesting results. The Pro12Ala polymorphism of the PPARG gene, which is known to be protective against diabetes in Europeans, does not appear to offer protection to Indians. We also observed that the Thr394Thr (G > A) polymorphism of PPARGC1A gene to be strongly associated with T2D and with body fat in Indians which has not been reported in other ethnic groups. The Gly1057Asp of IRS2 gene predisposes Indians to diabetes particularly in the presence of obesity. Subjects with TCF7L2 gene G > T polymorphism at rs12255372 showed 1.5-fold higher risk of having diabetes confirming similar association in other populations. Our studies on the MODY 3 gene, HNF1A gene yielded interesting results. The Ala98Val showed an association with earlier age at onset of type 2 diabetes. We have also carried out genetic studies on monogenic forms of diabetes. Our work on MODY resulted in identification of novel mutations in HNF4A (MODY1) and HNF1A (MODY3) genes. Co-segregation in large pedigree has also shown novel MODY mutations in south Indian population. Our studies on KCNJ11 gene in neonatal diabetes have also yielded important results which will help in planning treatment modalities in such patients. More large-scale and in-depth genetic studies are underway which will help in determining subjects with high risk for diabetes and in planning prevention strategies.