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ABSTRACTS

Genes, chromosomes and disease

376: Genetic variations in endothelin-1 but not in endothelial nitric oxide synthase and angiotensin converting enzyme are associated with preeclampsia

[1]**Pardeep Kumar Aggarwal**, [2]Vanita Jain, [1]Vivekanand Jha

[1]Department of Nephrology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [2]Department of Obstetrics and Gynaecology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Preeclampsia (PE), a hypertensive pregnancy specific syndrome is characterized by development of new-onset hypertension, proteinuria and edema. Endothelial dysfunction involving imbalance in endothelium derived vasoactive factors has been shown crucial to PE manifestations. We hypothesize that variation in endothelial vasoactive elements encoding genes such as endothelial nitric oxide synthase (eNOS, encoded by NOS3, produces a vasodilator nitric oxide), angiotensin-1 converting enzyme (ACE, generates vasoconstrictor angiotensin-II) and endothelin-1 (ET-1, encoded by EDN1, a potent vasoconstrictor) may contribute to endothelial dysfunction and hence PE. In this study, a total of 324 subjects were recruited and the frequencies of eNOS Glu298Asp, 4B/A and T-786>C, ACE I/D and ET-1 Lys198Asn polymorphism were assessed using PCR-RFLP method in preeclamptic (PE), gestational hypertensive (GH) and normotensive (N) pregnant women. No difference was observed in the distribution of Glu298Asp, 4B/A and T-786>C and ACE I/D polymorphism genotypes among the three groups, both at genotypic and allelic levels. In contrast, 198Asn variant of ET-1 Lys198Asn polymorphism was found to be more frequent at genotypic [OR (PE v GH+N): 1.58; OR (GH v N): 2.84] and allelic [OR (PE v GH+N): 1.34; OR (GH v N): 2.09] levels in PE and GH groups. In conclusion, our study suggests that eNOS and ACE polymorphism do not alter the risk of PE in Indian women but women homozygous or even carrier for the ET-1 198Asn allele are at increased the risk of developing hypertension, a preeclampsia phenotype.

377: Extensive alternative splicing of *Dmrt1* during gonadogenesis in Indian mugger, a species exhibiting temperature-dependent sex-determination

R. K. Aggarwal, Amit Anand, Minarbha Patel, Lalji Singh

Centre for Cellular and Molecular Biology, Uppal Road, Tarnaka, Hyderabad 500007, India

Dmrt1 is an evolutionarily conserved gene having important role in the sex determination from invertebrates to mammals. Recent studies show transcriptional diversity for this important gene during gonadal differentiation in a few vertebrate species having genetic sex-determination (GSD). In this study, we show for the first time that transcriptional diversity of *Dmrt1* is also well conserved in Indian mugger that exhibits temperature-dependent sex-determination (TSD). We report here isolation and characterization of eight novel isoforms of *Dmrt1* from *Crocodylus palustris*, along with its genomic locus, cpDmrt1. We demonstrate that all the isoforms are generated by alternative splicing, exonization of intronic sequences and alternative polyA sites from the same locus. The eight transcripts range from 494 to 2,060 bp and encode six predicted proteins having the characteristic DM domain of *Dmrt1*. The major heterogeneity in the isoforms and their predicted proteins is seen only in their C-termini and 3′-UTRs, which do not match with any similar sequences reported for other vertebrates. The cpDmrt1 expression was seen mainly in developing GAM (genital ridge–adrenal–mesonephros complex) with significant upregulation only in male embryos from the start of the temperature sensitive period (TSP). More significantly, ~70% of this expression was contributed by only one isoform (*cpDmrt1e*) that also has a unique 15 amino acids domain towards its C-terminal. *cpDmrt1* expression was also detected at a lower level in brain and developing kidney. The study thus provides the first account of Dmrt1 locus, regulation of its transcriptional diversity and sex-specific expression in a TSD species.

378: Centre for Arab Genomics Studies: current focus and future direction

Mahmoud Taleb Al Ali

Centre for Arab Genomic Studies, P.O. Box 22252 Dubai, United Arab Emirates

Since its inception in 2003, scholars at the Centre for Arab Genomic Studies (CAGS) realized that progress in understanding the etiology of genetic disorders in the region comes in little steps that will add up to one big leap forward. One of those first little steps achieved in CAGS was the successful launch of its Catalogue of Transmission Genetic in Arabs (CTGA) database, which is a continuously updated

compendium of bibliographic material and observations on human gene variants and inherited, or heritable, genetic diseases in Arab individuals. Using a comprehensive search strategy in international and national peer-reviewed medical journals, it was possible to bring together information on the presence of more than 900 genetic disorders in the region. Early in 2005, CAGS made another practical step by exploring the molecular pathology leading to an inherited skeletal abnormality in a UAE family. Initial results of this study have been recently published and work is currently ongoing to depict the gene mutation responsible for this disease. The genetic depiction for this disorder will surely bring fresh insights to our understanding of the human genome and will pave the way for many similar projects in light of the presence of many non-characterized inherited disorders in Arab populations. Another significant step achieved was the formation of the Arab Council of CAGS early in 2006 with the aim to lay the foundation of a regional network to support present and future activities of the centre. It is by way of the Arab Council that CAGS will achieve a thorough understanding of the spectrum of genetic disorders in the region. For this reason, CAGS started working closely with nuclear groups of scientists in 12 Arab countries to conduct local projects of data collection. In 2005, data collection from the United Arab Emirates succeeded in cataloguing 240 genetic disorders. In 2006–2008, data from Bahrain and Oman indicated the presence of nearly 109 and 271 genetic disorders, respectively. Current efforts are being conducted to define the spectrum of genetic disorders in Qatar.

379: Evolution of human trifunctional GART gene and identification of elements important for gene transcription in neurodevelopment

D. Banerjee, K. Nandagopal

Manovikas Biomedical Research and Diagnostic Centre, Manovikas Rehabilitation and Research Institute for the Handicapped, 482 Madudah, Plot I-24, Sector J, Eastern Metropolitan Bypass, Kolkata 700107, India

Background: The GART gene which encodes a fused tri-functional GARS-AIRS-GART enzyme protein involved in de novo purine biosynthesis, is implicated in Down syndrome (DS)-related neurodevelopmental delay due to its chromosomal location (21q22.1), over-expression in DS cerebellum and increased serum purine catabolite levels in DS patients.
Aim: This study addresses evolution of the fused GART sequence and identification of elements important for regulation of gene transcription in neurodevelopment.
Methods: Based on bootstrap values and genetic distance estimates, phyletic relationships were traced through rooted Neighbour-Joining, Maximum-likelihood and Parsimony tree construction using orthologous genomic DNA and protein sequences of GART. Identification of promoter, CpG island and transcription factor binding sites was performed with Gene2Promoter, CpG plot/CpG and MatInspector software programs, respectively. Functional role(s) of the identified transcription factors was ascertained from survey of published literature databases. Tissue-specific expression of the transcription factors was determined by mining human (T1Dbase, Unigene), murine (GXD) expression databases.
Results: Genetic distance and bootstrap value estimates indicate that murine and bovine orthologues are closest relatives of human GART. The promoter harbours 1 CpG island in each of human, murine, bovine orthologues. We identified binding sites for Brn3, Pax3 and Otx2 transcription factors. Literature survey reveals functional role(s) for Otx2 in eye and cerebellum; Brn3 in eye and inner ear; Pax3 in skeletal myogenesis and inner ear. Expressed sequence tag database (UnigeneEST) search shows that Otx2 and GART transcripts are expressed at high levels in human and murine eye. The T1Dbase collection of microarray, SAGE and EST data, reveals co-expression of Otx2 and GART in human cerebellum. RNA in situ hybridization data from GXD database reveals expression of Otx2 in cerebellum of P7, 15, 21 mice. The co-expression of GART and Otx-2 in eye and cerebellum, involvement of de novo purine biosynthesis in eye defects of *Drosophila melanogaster* mutants, is reminiscent of visual and postural defects in DS patients, suggesting potential interaction(s) between GART and Otx2 in DS-related sensory and motor development.
Acknowledgements: DB is recipient of JRF from CSIR grant 27(0131)/04/EMR-II awarded to KN.

380: Gene–gene interaction between SLC6A4 and HTR1B contributing to the risk of ADHD in Indian population

[1]**Emili Banerjee**, [2]Swagata Sinha, [2]Anindita Chatterjee, [1]Krishnadas Nandagopal

[1]Manovikas Biomedical Research and Diagnostic Centre, Manovikas Reh, 482 Madudah, Plot-I-24, Sector-J, Eastern Metropolitan Bypass, Kolkata 700107, India, [2]Manovikas Kendra Rehabilitation and Research Institute for the Han, 482 Madudah, Plot-I-24, Sector-J, Eastern Metropolitan Bypass, Kolkata 700107, India

Background: The serotonin transporter (SLC6A4) and serotonin receptor 1B (HTR1B) genes mediate susceptibility to Attention-deficit hyperactivity disorder (ADHD). As ADHD is polygenic and shows complex inheritance, gene × gene and gene × environment interactions are part of the etiology. Prior studies from our laboratory have shown that the STin2.12 allele of SLC6A4 and the C861 allele of HTR1B are both associated with and linked to ADHD. More significantly, selective maternal inheritance of these alleles confers increased risk of disease. Objective: We asked whether there is a genetic interaction between these loci in the context of ADHD and developed a one-tube PCR-based genotyping test for the alleles. The genotype of ADHD cases was compared with the behavioral phenotype, as determined by the Conner's Rating scale.
Methods: A total of 86 ADHD patient families were included in the analysis. The gene–gene interaction component was estimated with the help of two nonparametric statistics, namely the Extended Multifactor Dimensionality Reduction (EMDR) and Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT). Using sex of the ADHD individuals as phenotypic covariates we analysed the environmental effect on gene–gene interaction with the help of MDR-Phenomics. Using the two sets of primers specific for STin2 and G861C we set up a single tube reaction for PCR-RFLP based genotyping. Conner's rating score was determined for 52 patients using a structured questionnaire.
Result: The EMDR statistics reveals significant probability ($\chi^2 = 11.15$, $p = 0.03$, 95% CI) of interaction between these two loci with increased risk of ADHD. The MDR-PDT statistics ($\chi^2 = 3.206$, $p = 0.04$, 95% CI) also indicates positive association between C861 and STin2.12. The M statistic, given by $M = C \times F$, is a measure of interaction between allele transmission status associated with genotype (C statistic) and the effect of the phenotypic co-variates on this association (F statistic). We estimated $M = 28.72$, $p = 0.022$, 95% CI, which is statistically significant. The distribution of genotype frequencies is not directly correlated with the distribution of Conner's Rating Scores.
Conclusion: The STin2.12 and 861C alleles of SLC6A4 and HTR1B genes confer susceptibility to ADHD, both individually and jointly; cases may be genotyped by single tube PCR. In addition to gene × gene interaction between serotonergic genes, other systems and environmental influences also regulate susceptibility to ADHD.

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381: CompreSNPdb: comprehensive data-mining workflow for SNPs, genes, diseases and pathways

[1]**Anshu Bhardwaj**, [2]Ajeet Pandey, [2]P. Krishna manjari, [2]V. Kiran Kumar, [2]Nita Parekh, [1]Shrish Tiwari

[1]Centre for Cellular and Molecular Biology (CCMB), E503, Bioinformatics Division, CCMB, Hyderabad 500007, India, [2]International Institute of Information Technology (IIIT), IIIT, Gachibowli, Hyderabad 500032, India

A common task in disease gene association studies and pharmacogenomics research involves retrieving information on human diseases, which includes genes associated to diseases, variations reported, their frequency in different populations, effect on protein structure, functional implications, biological pathways affected, etc. Each of this information is spread across various specialized databases, e.g. databases KEGG and HumanCyc focus on cellular pathways, OMIM, GAD and GATACA are archives of disease related information, dbSNP, HapMap, etc. focus on SNPs and their population statistics. Retrieving comprehensive information for a specific query (gene, pathway, SNP, etc.) is a difficult task, as the data are fragmented and stored in multiple sources, not necessarily inter-connected. CompreSNPdb has been designed with the objective to facilitate the data-mining task by integrating information from independent resources to provide useful insight into genes and variations associated to human diseases. Currently, it integrates information from 17 databases, viz., Entrez gene, dbSNP, SNP500cancer, SNPs3D, HapMap, Ensembl, ALFRED, SNP2NMD, GO, GOA, LSDBs, OMIM, GAD, GATACA, KEGG, HumanCyc and Inparanoid Eukaryotic Ortholog Groups. CompreSNPdb has three components, namely, Local database, Web interface and Search modules. The local database is created in MySql (Version 5.0.27) and the interface and search modules are developed using PHP (Version 5.1.6). The search is performed on the local databases and for updated information, links are provided for most of these databases in addition to links to SNP2NMD, Uniprot and Pubmed. There are four search modules, namely, gene, disease, pathway and SNP. These modules support fuzzy match searches so that the user is not restricted to enter the exact gene identifiers/symbols or disease names to extract relevant information. Besides, various filtering options are provided for customized retrieval of information. We have demonstrated the utility of CompreSNPdb to test predictions made by other tools and in generating a testable hypothesis based on the information retrieved by the search modules. CompreSNPdb's unique querying features coupled with its user-friendly interface allow the user to search heterogeneous databases and obtain customized and relevant information for SNPs, genes, diseases and pathways. CompreSNP thus is expected to be helpful in designing focused laboratory experiments.

382: Mutation in ATPase gene regulating oxidative phosphorylation in sperm mitochondria and low seminal antioxidant levels in idiopathic asthenozoospermic men

[1]**A. Bhat**, [2]R. Kumar, [2]S. Venkatesh, [2]M. Kumar, [2]R. Dada

[1]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India, [2]Laboratory of Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Science (AIIMS), New Delhi 110029, India

Approximately 15% of the married couples failed to achieve parenthood after 1 year of regular unprotected intercourse and sole male factor is responsible for at least half of these infertile cases. Approximately 40% of the male factor infertility is diagnosed as idiopathic. Seminal antioxidant enzymes, which maintain the physiological levels of ROS are lowered in known and unknown pathological conditions. Since mtDNA is the key genome for the production of energy in the form of ATP, they are also both source and target of ROS. Thus it is necessary to study the role of oxidative stress associated mtDNA mutation in idiopathic infertile men. The present study includes 40 infertile asthenozoospermic men and 30 proven fertile controls. Semen analysis was carried out according to WHO (1999) guidelines. Catalase, glutathione peroxidase, and malondialdehye (MDA) levels in the seminal plasma were estimated spectrophotometrically. DNA from spermatozoa were isolated from both cases and control and mitochondrial genome was amplified and sequenced by standard PCR-DNA sequencing protocol. Infertile men had significantly lowered sperm count (43.3 ± 4.1 vs. 62.1 ± 16.2) and percent progressive motility (11.8 ± 7.0 vs. 66.3 ± 10.4) than fertile controls. Semen MDA levels of infertile group was found to be significantly higher when compared to the control group. However, catalase, and glutathione peroxidase (GPx) levels were significantly lower (239.0 ± 11.96 vs. 307.5 ± 15.29 U/l and 120.6 ± 8.9 vs. 166.3 ± 11.47 U/mg protein, respectively) in infertile men compared to control men. Mitochondrial DNA (mtDNA) sequencing showed high frequency of nucleotide changes in the ATPase 6 (8279, 8280, 9098 and 8394) and ATPase 8 (8701, 8860 and 8879) genes of infertile men as compared to controls. Hence increased lipid peroxidation and low antioxidant levels induced oxidative stress coupled with ATPase mutation in OXPHOS pathway may be involved in impaired motility in such cases. Thus chronic exposure of the sperm to the high levels of ROS and lipid peroxidation may lead to irreversible changes in the mitochondrial and nuclear DNA. Thus early diagnosis of low antioxidant levels and oxidative stress and prompt management with antioxidants may prevent irreversible DNA damage in such cases.

383: Interleukin 1 Beta as potential host susceptibility factor in *Helicobacter pylori* mediated duodenal ulcer in eastern Indian population

[1]**Sumana Bhattacharjya**, [1]Dipanjana Datta De, [2]Meenakshi Maitra, [1]Subhabrata Purakayastha, [3]Abhijit Choudhury, [3]Susanta Roychoudhury

[1]Molecular and Human Genetics Department, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick, Kolkata 700032, India, [2]Department of Pediatrics and Molecular Biology, UT Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas TX 75390, United States of America, [3]Department of Medicine, Department of Gastroenterology, Institute of Postgraduate Medicine and Experimental Research, 244, A. J. C. Bose Road, Kolkata 700020, India

Helicobacter pylori is a gastric pathogen that chronically infects more than half the world population. *H. pylori* infection and associated gastric diseases are common in developing countries including India. The majority of the infected individuals do not develop any clinically apparent disease but there is compelling evidence that 6–20% of the infection results in duodenal ulceration and a smaller proportion is associated with gastric cancer. Genetic predisposition to differential clinical outcomes upon *H. pylori* infection has been suspected over a long period of time. IL1B -511T>C and -31T>C

promoter polymorphisms have been previously reported as crucial markers in the genetic predisposition to duodenal ulcer upon *H. pylori* infection. However, recent evidence suggests presence of two additional functional SNPs at -1464G>C and -3737C>T loci also affect IL1B gene expression due to differential binding of transcription factors. In the present study, 250 individuals from Eastern India were subjected to a case–control study to determine the IL1B risk genotypes at -1464G>C and -3737C>T loci in *H. pylori* mediated duodenal ulcer. An analysis of genotype frequency revealed a higher frequency of IL1B -3737 allele in *H. pylori* infected individuals with duodenal ulcer compared to infected individuals with normal mucosa. Association study with -1464G>C locus suggested the G/G genotype to be protective (OR = 0.286, 95% CI = 0.136–0.680, p = 0.036). Extended haplotype analysis using all four loci will be discussed.

384: NF-kB signaling pathway in acute leukemia: a study on expression of cell survival and proliferative genes by real time RT-PCR

[1]**Bharat Bhushan**, [1]Pradeep Singh Chauhan, [2]Sumita Saluja, [1]Ashwini Kumar Mishra, [2]Sumita Bhasin, [2]Dipendra Kumar Gupta, [3]Saeed Siddiqui, [1]Sujala Kapur

[1]Institute of Pathology (Indian Council of Medical Research), Safdarjung Hospital Campus, New Delhi 110029, India, [2]Division of Hematology and Department of Medicine, Safdarjung Hospital, New Delhi 110029, India, [3]Department of Medical Elementology and Toxicology, Jamia Hamdard, New Delhi 110062, India

Background: NF-kB signalling is known to be aberrantly activated in acute leukemia (AL) making it a suitable target pathway for therapeutics. Several inhibitors of the NF-kB pathway, including common synthetic (e.g. aspirin) and traditional (e.g. green tea, curcumin) remedies, have been identified and some inhibitors are entering clinical trials. However, molecular pathways involved in NF-kB dysregulation remain controversial. Knowledge of the signaling pathways in NF-kB-activated samples with AL may enable the development of more specific and potent inhibitors that not only enhance the efficacy of anticancer treatments but may also be helpful in designing individualized therapy.
Aim: To find out if transcriptional activation of genes whose products help in cell survival (BCL2, BIRC2 and Survivin) or cell proliferation (Cyclin D1) is associated with NF-kB activation in samples with AL.
Material and methods: RNA isolated from ALL and AML blasts were reverse transcribed to c-DNA. Expression of selected genes of NF-kB pathway including BCL2, BIRC2 (BIRC2), BIRC5 (Survivin), CCND1 (Cyclin D1) and IKBKB (Ik-B) were analysed by Real Time RT-PCR using TaqMan assays. Expression of Ik-B was used as a reporter of activation of NF-kB pathway. Normal bone marrow was used as a control.
Results: NF-kB pathway was found to be activated in 67 and 88% cases of ALL and AML respectively. Expression of Ik-B (reporter of NF-kB activation) was significantly higher in AML than ALL blasts (7.7 ± 9.3 and 2.2 ± 0.3-fold respectively, p = 0.03). Expression of cell survival genes i.e. BCL2 and BIRC2 was found to be upregulated in both ALL (2.0 ± 0.5 and 2.5 ± 0.9-fold) and AML (2.3 ± 2.3 and 4.6 ± 3.8-fold). However, expression of survivin was downregulated in ALL (0.2 ± 0.05) and AML (0.1 ± 0.09). There was no statistical difference in the expression of BCL2 and BIRC2 genes in ALL and AML (p = 0.7 and p = 0.06 respectively). Downregulaton of survivin was significantly more in AML as compared to ALL (p = 0.02). Expression of cyclin D1, cell proliferative gene, was found to be marginally downregulated in both AML (0.4 ± 0.4) and ALL (0.6 ± 0.6), and statistically no difference was found between the groups (p = 0.7).
Conclusion: Samples of AML were significantly more likely to have activated NF-kB pathway as compared to ALL. The dysregulation of NF-kB signaling was associated with upregulation of cell survival genes rather than cell proliferative genes in both ALL and AML indicating that targeted therapy to downregulate cell survival genes may be beneficial in these cases.

385: Evaluation of PINK1 variants in Indian Parkinson's disease patients

[1]**Arindam Biswas**, [1]Tamal Sadhukhan, [1]Sayantani Majumder, [2]Shyamal K. Das, [3]The Indian Genome Variation Consortium, [4]Kunal Ray, [1]Jharna Ray

[1]S. N. Pradhan Centre for Neurosciences, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700 019, India, [2]Bangur Institute of Neurosciences and Psychiatry, 52/1-A, Sambhunath Pandit Street, Kolkata 700 025, India, [3]Nodal Laboratory, Institute of Genomics and Integrative Biology (CSIR), Near Jubilee Hall, Mall Road, Delhi 110 007, India, [4]Molecular and Human Genetics Division, Indian Institute of Chemical Biology (CSIR), 4, Raja S.C. Mullick Road, Kolkata 700 032, India

Mutations in the PINK1 gene have been reported to be causal to autosomal recessive early onset Parkinson's disease (PD), and recently detected in sporadic early onset Asian PD patients as well. To determine the prevalence of PINK1 mutation in Indian PD patients, we conducted genetic analysis of PINK1 mutation in 250 PD patients with mean age of onset 49 ± 12 years and 105 ethnically matched controls from eastern India. PINK1 mutations were screened by polymerase chain reaction, single-stranded conformation polymorphism and DNA sequencing. A total of 15 changes were identified, among which eight were found in exons and the other seven in introns. These include two novel missense mutations (Arg246Gln and Arg276Gln) detected in five patients in heterozygous state. The only non-synonymous cSNP, Ala340Thr did not show any statistical bias in distribution of any specific allele or genotype frequency between cases and controls. In a parallel effort, six SNPs spanning the entire gene was genotyped in 531 individual representing 24 different ethnic groups of India (J Genet 87:3–20, 2008) by allele-specific primer extension followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Most of SNPs were observed to be highly heterozygous in Indian and the HAPMAP populations, except Yoruba, and could be used as markers to examine cosegregation of PINK1 with PD in familial cases. Among the eastern Indian PD patients, mutation in the PINK1 was identified in 2% cases which need to be further investigated on additional cohort of patients. The study was funded by a grant from Council of Scientific and Industrial Research, Government of India.

386: Chromosomal aberrations and micronuclei as biomarkers of arsenicosis; a study in an arsenic exposed population in West Bengal, India

T. Chakraborty, M. De

Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, 99 Sarat Bose Road, Kolkata 700026, India

Background: Arsenic is a paradoxical human carcinogen. In West Bengal it affects around 42.7 million people. Arsenicosis is the effect of arsenic poisoning due to high level of arsenic in body, usually over a long period such as from 5 to 20 years.
Materials and methods: A total of 100 arsenic exposed individuals and 50 age and sex matched healthy controls were taken for this study. The exposed individuals were divided into 3 groups based on their year of exposure. The individuals of group 1 were exposed for <20 years, group 2 was exposed for 21–30 years and group 3 was exposed for more than 30 years. Arsenic from water, hair and nail samples of all exposed group and control group individuals were studied. Oral mucosal cell was collected from each subject to study MN from buccal smear and peripheral blood was collected to study CA and MN from blood.
Results: In exposed individuals the mean(±SD) arsenic content in the drinking water was found to be 66.75 ± 2.50 μg/l which was above the WHO recommended level of 10 μg/l. The mean(±SD) arsenic content in hair and nails of the exposed individuals was found to be, 1,871.87 ± 709.56 μg/kg and 2135.48 ± 984.55 μg/kg respectively which was significantly higher ($P < 0.01$) than the control. In the present study we found that in the individuals belonging to exposure group 1, the mean(±SD) CA percentage was 5.17 ± 1.71, buccal smear MN percentage was 0.83 ± 0.33, and blood MN percentage was 0.43 ± 0.17. Whereas, in the individuals belonging to exposure group 2, the mean(±SD) CA percentage was 5.61 ± 1.58, buccal smear MN percentage was 0.91 ± 0.36, and blood MN percentage was found to be 0.49 ± 0.11. In comparison, the individuals belonging to exposure group 3 the mean(±SD) CA percentage was 6.44 ± 1.47, buccal smear MN percentage was 0.93 ± 0.28, and blood MN percentage was 0.51 ± 0.14. All these are significantly higher ($P < 0.01$) than the control group where the mean(±SD) CA percentage was 0.86 ± 0.76, buccal smear MN percentage was 0.29 ± 0.07, and blood MN percentage was 0.11 ± 0.06 respectively. It was visible from this study that increase in MN and CA percentages were proportional with the year of exposure.
Conclusion: Chromosomal aberrations and micronuclei can serve as an efficient biomarker of arsenic exposure.

387: GST polymorphism, micronuclei frequencies-risk markers for oral cancerous lesions and their prevalence in other oral mucosal lesions

Sumana Chatterjee, Sila Chakrabarti, Bani Sengupta, Soujatya Dhar, Asish Ghosh, Manas De, S. Roy, Geeta Talukder

Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, 99, Sarat Bose Road, Kolkata 700 026, West Bengal, India

Background: Oral cancer, a malignancy of lip, mouth or tongue is the most common cancer in males and third most common in females in India. Environment–gene interaction in oral carcinoma is well explained by Phase I and Phase II enzymes, involved in the metabolism of carcinogens. The study investigated the association of different polymorphic genotypes of glutathione-S-transferase, a phase II enzyme. The presence of micronuclei reflects genotoxocity and it has been used as an important marker of genotoxic marker. The study included the clinically diagnosed cases of oral carcinoma and other oral mucosal pathology. All the patients were ethnically same, the Bengali, and were chosen randomly.
Method: Cases were chosen with oral cancer ($N = 50$) and with other oral pathology ($N = 30$). The detailed history of their tobacco intake was noted. 50 age and sex matched controls were chosen. DNA was extracted from the peripheral blood. PCR was done followed by agarose gel electrophoresis for the study of different GST polymorphisms. Micronuclei frequencies were scored from buccal mucosal cells to monitor the extent of DNA damage. Results: DNA studies detected that 54% of the cases were having normal GSST genotype while 32% had GSTM1 null and 10% had GSTT1 null genotypes. 4% of the cases were found to have both GSTM1 and GSTT1 null genotypes. Significant association ($p < 0.50$) was found between tobacco exposure and development of oral cancer after performing chi-square test. Association studies reveal that GST null genotypes are less significantly associated with other oral pathology ($p < 0.10$) than cancer cases ($p < 0.001$). But prevalence of micronuclei frequencies is quite same in both the cases, which is 2.31 and 2.03% respectively that is indicative of mucosal damage in both the cases.

388: Differential expression of MAPK and GPCR pathway in esophageal cancer of North-east region of India

[1]**Indranil Chattopadhyay**, [1]Avninder Pal Singh, [1]Sujala Kapur, [2]Rupkumar Phukan, [3]Joydeep Purkayastha, [2]Jagadish Mahanta, [4]Hewitt Stephen, [3]Jaganath Sharma, [1]Sunita Saxena

[1]Institute of Pathology, (Indian Council of Medical Research), Safdarjung Hospital Campus, P.O. Box 4909, New Delhi 110029, India, [2]Regional Medical Research Center, Dibrugarh 786001, Assam, India, [3]Dr. B. Borooah Cancer Institute, Guwahati 781016, Assam, India, [4]TARP Lab, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States of America

Background: The development of esophageal cancer is a leading example in which environmental carcinogens in addition to geographic and genetic factors appear to play major etiologic roles. The highest incidence of this cancer in India has been reported from Assam in the North-east region where it is the second leading cancer in men and third leading cancer in women. Tobacco smoking, betel quid chewing, and alcohol consumption are the major known risk factors for esophageal cancer in Assam.
Objective: To identify alterations in genes and molecular functional pathways in esophageal cancer in a high incidence region of India where there is a widespread use of tobacco and betel quid with fermented areca nuts.
Methods: Total RNA was isolated from tumor and matched normal tissue of 16 patients with esophageal squamous cell carcinoma (ESCC). Pooled tumor tissue RNA was labeled with Cy3-dUTP and pooled normal tissue RNA was labeled with Cy5-dUTP by direct labeling method. The labeled probes were hybridized with human 10K cDNA chip and expression profiles were analyzed by Genespring GX V 7.3 (Silicon Genetics). Functional genes were validated by real-time PCR and Tissue Microarray in 60 ESCC patients' samples.
Results: Using stringent criteria, 127 differentially expressed genes (87 up regulated and 40 down regulated) were identified in tumor tissue. On the basis of Gene Ontology, four different molecular functional pathways (MAPK pathway, GPCR family, ion transport activity, and serine or threonine kinase activity) were most significantly up regulated and six different molecular functional pathways (structural constituent of ribosome, endopeptidase inhibitor activity, structural constituent of cytoskeleton, antioxidant activity, acyl group transferase activity, and eukaryotic translation elongation factor activity) were most significantly down regulated.
Conclusion: NPY (7p15.1) involved in G-protein coupled receptor activity and FGF12 (3q28) involved in MAPK activity were significantly up-regulated in tobacco and betel quid associated esophageal malignancies at mRNA and protein level. It is possible that ingestion

of genotoxic chemicals of tobacco and betel quid induce the release of inflammatory mediators and uncontrolled proliferation through activation of MAPK and GPCR family.

389: Immunophenotypic and clinical findings in adult acute myeloid leukemia with FLT3 internal tandem duplication

[1]**Pradeep Singh Chauhan**, [1]Bharat Bhushan, [1]Saurabh Verma, [2]Sumita Saluja, [2]Monica Sharma, [1]Ashwini kumar Mishra, [2]Vishakha Mittal, [2]Meghana Kabra, [1]Sujala Kapur

[1]Institute of Pathology (Indian Council of Medical Research), Safdarjung Hospital Campus, New Delhi 110029, India, [2]Division of Hematology and Department of Medicine, Safdarjung Hospital, New Delhi 110029, India

Introduction: Most acute myeloid leukemia (AML) patients are intrinsically resistant to chemotherapy. Activating mutations on FLT3 receptors are one of the most common genetic alterations in AML, conferring poor prognosis. The FLT3 gene is mutated either by internal tandem duplication (ITD) of the Juxtamembrane domain (occurs in about 20% of AML cases) or by activating point mutation in the tyrosine kinase domain. Mutation in FLT3 is believed to promote its autophosphorylation and constitutive activation, leading to ligand-independent proliferation. Elevated white blood count is one of the most significant negative prognostic factor associated with the presence of FLT3/ITD mutation.
Aim: The aim of this study was to evaluate the immunophenotypic and clinical characteristic of internal tandem duplication in the JM domain of the FLT3 gene in adult AML patients.
Material and methods: Immunophenotyping studies were performed at diagnosis on blood/bone marrow samples from 71 adult AML patients using labeled antibodies. The genomic DNA polymerase chain reaction assay was performed to detect the FLT3/ITD mutation.
Results: FLT3/ITD mutation was found in 27% (19/71) cases of adult AML patients. Among the FAB subtypes of AML, M2 showed higher frequency for FLT3/ITD+ (42%). Immunophenotypic analysis of FLT3/ITD+(mutant) and FLT3/ITD−(wild) cases showed no difference for CD7 positivity. Expression of CD34 (immature stem cell marker) also showed no difference between FLT3/ITD+ (63%) and FLT3/ITD− (60%) cases. Correlation study showed no significant association between FLT3/ITD+ and FLT3/ITD− for hematological and clinical features except lymphadenopathy which was statistically significant ($p = 0.029$). No difference observed for the clinical response between FLT3/ITD+ and FLT3/ITD−.
Conclusion: FLT3/ITD is preferentially associated with more of immature FAB subtypes (M2). The study displays that there was no difference in the expression pattern of adverse prognostic antigen such as CD34 and CD7 between FLT3/ITD+ and FLT3/ITD−.

390: Genome wide search for novel cancer gene in familial adenomatous polyposis variant patients without detectable APC mutation

Peh Yean Cheah, Lai Fun Thean, Carol Loi, Kong Weng Eu

Department of Colorectal Surgery, Singapore General Hospital, Singapore

Background and aim: FAP is an autosomal dominantly inherited form of colorectal cancer caused by a germline mutation in the adenomatous polyposis coli (APC) gene. We search for new cancer gene in a subgroup of APC mutation-negative FAP variant patients with autosomal dominant inheritance and typical adenomatous polyps (100s–1,000) but have accelerated disease progression.
Methods: We performed Whole Genome Sampling Assay on both the lymphocyte and polyp DNA of two affected individuals of a FAP variant family and lymphocyte DNA of 2 other affected, 4 unaffected members and 18 ethnicity-matched healthy controls using Affymetrix Genome-Wide Human single nucleotide polymorphism (SNP) Array 5.0. This SNP Array 5.0 enables genotyping of approximately 450K SNPs and assaying of an additional 500K non-polymorphic copy number (CN) probes, covering the entire genome with an average inter-probe distance of $\sim$10 kb.
Results: By using the Copy Number Tool of Partek Genomic Suite Software, a 40 kb region consisting of 17 CN but no SNP probes showed gain of copy number in germline of all four affected members (mean $CN = 4.20$) compared to the four unaffected members as baseline. We are currently verifying this copy number gain and investigating the expression of a putative oncogene 18 kb downstream. Further, a 111 kb region on another chromosome, consisting of 44 CN probes, showed copy number loss in all 8 polyp DNAs (mean $CN = 0.68$) compared to the matched lymphocyte DNAs of two affected individuals. Fragments chosen randomly from this critical region for PCR analysis further confirmed the copy number loss. Data mining with NCBI Build 36.2, however, reveal no known gene but only one putative 2.2 kb transcript in melanoma tissue. Extensive RT-PCR analysis indicated that this transcript is probably non-existent in lymphocyte and colonic mucosa tissues. Conclusion: Structural variations may contribute to polyposis in FAP variant patients without detectable APC germline mutation.

391: Modelling potential CNV effects on human fertility by manipulating meiosis in mice

Howard J. Cooke, Evelina Bolcun-Filas, Robert Speed, Mary Taggart

MRC Human Genetics Unit and Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, United Kingdom

Male and female fertility requires completion of meiosis to produce the haploid gametes. Meiosis is a complex process in which the two genomes in the diploid cell have to be aligned with single base pair accuracy and linked by crossover events to ensure correct segregation at the cell divisions needed to produce haploid cells. In humans fertility is highly variable and a substantial part of that variation is genetic. The phenotype is hard to measure and involves input from two individuals making whole genome association studies difficult if not impossible. Candidate gene approaches based on a knowledge of the underlying processes of gametogenesis have had limited success. This is a reflection of the large number of genes thought to be required, particularly for sperm production, and since the majority of mutations are likely to be recessive, homozygosity at any one locus for such a mutation is likely to be rare. It is possible that more common processes such as copy number variation may be involved. The level of proteins in the cell may be critical of function especially when forming structural complexes with multiple other proteins. We have asked if compound heterozygosity at genes encoding proteins required for meiosis can affect the process. SYCE1 and SYCE2 are proteins of the meiotic Synaptonemal Complex. We have shown that SYCE2 is essential for male and female fertility in mice and here we show that this is also true for SYCE1. Heterozygous null animals are fertile in the case of both genes. Male mice heterozygous for both null alleles are also fertile but have an increased rate of XY asynapsis and reduced sperm count showing that compound hemizygosity in genes involved in the same pathway can have a significant impact. Since

these proteins form a complex with others to generate the Synaptonemal Complex without which cross-overs can not complete it is likely that the defects we observe result from changes in protein levels. Copy number variation could result in similar effects in human populations.

392: Analysis of sperm and blood mtDNA mutations in infertile oligoasthenozoospermic men

[1]**R. Dada**, [1]R. Kumar, [1]S. Venkatesh, [1]M. B. Shamsi, [2]R. N. Bamezai, [3]R. K. Sharma, [4]R. Kumar, [4]N. P. Gupta

[1]Laboratory of Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India, [2]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India, [3]Assisted Reproductive Technique Centre, Army Research and Referral Hospital, New Delhi 110010, India, [4]Department of Urology, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India

Sperm mitochondria are source of energy (ATP) for spermatogenesis and sperm motility by oxidative phosphorylation (OXPHOS) pathway. mtDNA is highly susceptible to free radical mediated damage and also accumulate mutation due to a very basic repair mechanism and lack of protamines. Since the number of mtDNA in male germ cell are very few as compared to somatic cell, we studied the frequency of mutation in blood (somatic) and sperm cells and to also determine which is ideal to study in understanding pathogenesis of OA. The study included 45 infertile patients with less than 20% progressive motile and less than 20 million sperm/ml in the semen and 30 age and ethnically matched fertile controls (who have initiated a successful pregnancy in the last 12 months). Semen analysis was performed according to WHO (1999) guidelines. Both blood and sperm DNA were isolated and sequenced for the mitochondrial genes (ND, Cyt b, CO I, and ATPase) by standard PCR-DNA sequencing protocol. More number of nucleotide changes were detected in the sperm mtDNA than the blood mtDNA of the OA patients. An average of 11.1 nucleotide changes were observed in the mtDNA of sperm compared to the mtDNA of the blood (6.6) for 10 nucleotides [7028 (MT-CO1), 8279, 8280, 8701, 8860 (MT-ATP6), 12612, 12705 (MT-ND5), 15043, 15226, 15257(MT-CYB)]. Based on our results we conclude that frequency of nucleotide changes are more in the germ cells compared to the somatic cells. Thus molecular screening of germ cell mtDNA is a better diagnostic marker than somatic cells to understand the etiology of oligoasthenozoospermia and counsel these men before they opt for ICSI.

393: Mutational analysis in beta-thalassaemics in Bhopal

[1]**C. B. S. Dangi**, [2]N. C. Sharma, [3]S. Gupta

[1]Human Genetic Reseach Centre, R and D Wing, People's Group, 4th floor, University Building, Bhopal, MP, India, [2]Department of Genetics, Barkatullah University, Hosangabad Road, Bhopal, MP, India, [3]Human Genetic Research cum counceling Centre, Jammu University, New Jammu University, Jammu, Jammu and Kashmir, India

Study report is based on identification by HPLC among 100 clinically suspected cases of haemoglobinopathies and identification of mutation in thalassaemics by ARMS among population of Bhopal. It was observed that Beta-thalassaemia trait ranks first in the list of haemoglobinopathies accounting for 26.23%, thalassaemia major (5.98%), thalassaemia intermedia (2.3%). The prevalence of total anemia was higher among the Beta-thalassaemia traits of this city. The reduced mean red cell indices indicate the hypochromic microcytic anemia among Beta-thalassaemia traits. All Beta-thalassaemia cases were confirmed by Genetic analysis on ARMS PCR. We find in our study that out of seven common Beta thal mutation (D. J. Weatherall & J. B. Clegg), IVS1 nt 5 (G-C), IVS1 nt 1 (G-T), 619bp deletion, Cap+1(A-C), 39.52, 16.27, 18.59, 6.97%, respectively found in population of Bhopal.

394: Gene expression profiling to understand the mechanism of action of enhancing factor in the Tg-K14EF transgenic mice

Poulami Das, Rita Mulherkar

ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai 410210, India

Enhancing Factor (EF) was first isolated in our laboratory from Balb/c mice intestine as a growth factor modulator. EF was found to increase the binding of Epidermal Growth Factor (EGF) by providing a binding site for EGF and thereby giving the cells a growth advantage. Sequencing data revealed that EF belongs to the non-pancreatic, secretory type II Phospholipase A2 (sPLA2) family. Transgenic mice expressing EF in squamous epithelial cells have been generated in our lab by pronuclear injection of EF cDNA sub-cloned under a human keratin14 promoter. Expression of EF, which coincided with hyperplasia and abnormal hair growth, was seen in squamous epithelial cells including tail skin, tongue, buccal mucosa and snout skin of these mice. We propose to study the differentially expressed genes in the skin of the Tg-K14EF mice in order to understand the hyperplastic phenotype seen in these mice and the role of EF in signal transduction. The expression of EF was confirmed in the tail, snout and tongue tissues of Tg-K14EF mice by immunohistochemistry and western blotting. In order to check for the differentially expressed genes, RNA was extracted from the tail, snout and tongue tissues of the transgenic as well as wild type littermates and a whole genome microarray was done using mouse 15K EST arrays from Toronto Microarray Centre. Analysis of the microarray data showed a number of genes to be differentially expressed in the transgenic mice as compared to the wild type control. The functions of these genes were found using databases like NCBI and KEGG pathway database. Out of all the genes, at this point the most significant one related our study appeared to be Lipocalin2 (Lcn2). This gene was found to be upregulated in the tail and snout tissues of Tg-K14EF mice and reports confirm that Lcn2 is induced by activation of EGFR. Apart from this, Lcn2 has also been reported as a marker and potential positive modulator of acute inflammation. The expression of this gene was checked at the protein level by western blotting whereby its expression was found to be much higher in Tg-K14EF skin than in wild type control.

395: Regulation of transcription by HIPPI and its molecular partner, Huntingtin Interacting Protein HIP1

[1]**Moumita Datta**, [1]Manisha Banerjee, [2]Pritha Majumder, [1]Nitai Bhattacharyya

[1]Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata 700064, India,

[2]Cell Stress Biology Department, Roswell Park Cancer Institute, Elm and Carlton Street, Buffalo, NY 14263, United States of America

HIP1 Protein Interactor (HIPPI) is known to increase apoptosis by recruiting pro-caspase8 into the HIPPI-HIP1 heterodimer complex and activating the enzyme and its downstream effector proteins. We have earlier shown that HIPPI can interact with specific sequence motif present at the putative promoter of caspase-1, caspase-8 and caspase-10. In the present communication, we shall present experimental evidences for the following: (1) Transcription regulation ability of HIPPI depends on R393 residue and R393E mutation affects transcription regulation ability, apoptosis induction ability, however, remaining unaffected. (2) Presence of HIP1 is necessary for the increase of caspase 1 transcription. (3) HIP1 is involved in translocating HIPPI into nucleus. Genome wide search for the presence of specific HIPPI binding motif observed earlier, reveals that ~10% of the promoters of ~23,000 genes in human genome harbour the motif. In addition, out of ~400 up-regulated and ~400 down-regulated genes in Huntington's disease, 17 genes harbour this motif. Experimental evidence that some of the genes are altered by forced expression of HIPPI is also obtained. Our preliminary data using microarray showed that HIPPI directly or indirectly can alter number of genes. Taken together, we have evidence that shows, HIPPI, without having any conventional DNA binding domain, can regulate transcription. Relevance of these results in connection with Huntington's disease pathogenesis will be discussed.

396: Investigation on angiotensin converting enzyme gene I/D polymorphism of Vitiligo in South Indian population

[1]**Farha Deeba**, [1]Roa Hanmanth Rao, [2]Kaiser Jamil, [3]Waheed M. Waheed

[1]Deapartment of Genetics, Osmania University, Hyderabad 500007, AP, India, [2]Indo American Cancer Institute and Research Centre, Banjara Hills, Hyderabad 500034, AP, India, [3]Central Research Institute of Unani Medicine, Department of Nuclear Medicine, Hyderabad, India

Vitiligo or leukoderma is a chronic skin condition that causes loss of pigment due to destruction of melanocytes, resulting in irregular pale patches of skin. Vitiligo is polygenic disease associated with autoimmunity. Angiotensin converting enzyme (ACE) is capable of modulating cutaneous neurogenic inflammation, hence this was the basis of this study. An insertion/deletion (I/D) polymorphism of a 287-base pair repetitive sequence in intron 16 of the ACE gene was reported to have been associated with autoimmunity and subsequent development of vitiligo. In our study, the distribution of ACE gene I/D genotype was investigated in a population of 100 South Indian vitiligo patients and 95 healthy controls using polymerase chain reaction genotyping method. The ACE gene allele frequency ($\chi^2 = 5.07(1df)$, $p = 0.02$) were significantly different between vitiligo patients and healthy controls. However no significant difference was detected in ACE gene genotype distribution ($\chi^2 = 4.27(2df)$, $p = 0.11$). This study suggests that the ACE gene allele frequency could confer susceptibility to vitiligo.

397: NPM1/FLT3 ITD gene mutation profiling in acute leukemia: a pilot study

[1]**Nageswara Rao Dunna**, [1]Sailaja Kagita, [1]Surekha Damineni, [2]Senthil Rajappa, [2]Raghunadharao Digumarti, [1]Vishnupriya Satti

[1]Department of Genetics, Osmania University, Hyderabad, India, [2]Department of Medical Oncology, Nizams Institute of Medical Sciences, Punjagutta, Hyderabad, India

Nucleophosmin 1 (NPM1) and internal tandem duplication of Fms-like tyrosine kinase 3 (FLT3/ITD) are some of the most frequent abberrations in patients with acute myeloid leukemia (AML). In the absence of FLT3/ITD, NPM1 mutations denote better response to induction therapy and favorable overall survival. FLT3/ITD alone and in combination with NPM mutations reflect unfavorable prognosis. To our knowledge, there are no existing data on NPM1 and FLT3/ITD mutations in Indian patients. Peripheral blood samples of newly diagnosed adult patients with acute leukemia were screened for the presence of NPM1 and FLT3/ITD mutations using polymerase chain reaction (PCR) followed by DNA sequencing ABI (3730). We tried to examine the correlation between incidence of NPM1, FLT3/ITD mutations and clinical and hematological prognostic factors in AML like age (< vs > 50 years), sex (male vs female), total leucocyte count TLC (< vs > 11,000 cells/mm^3), lactate dehydrogenase LDH (< vs > 400 U/l) and peripheral blast percentage (< vs > 50%). Fishers exact test with a 2 × 2 contingency table was used to calculate significance and a two-tailed p value of <0.05 was considered significant. Two hundred and forty cases of acute leukemia comprising of 114 cases of acute lymphoblastic leukemia (ALL) and 126 cases of AML were screened for mutations. NPM1 mutations alone were detected in 12 (9.5%) cases of AML and 1 (0.08%) patient of ALL. FLT3/ITD mutations were detected in 22 (17.4%) AML and 1 (0.87%) ALL patient. The incidence of NPM mutations was significantly higher in younger patients and those with lower LDH that correlate with better prognosis ($p = <0.0001, 0.03$). The incidence of FLT3 mutations was significantly higher in those with elevated LDH, TLC and peripheral blasts ($p = <0.002, 0.007, <0.001$). There was no difference in incidence of both mutations between sexes. All 10 (8%) AML patients who had both NPM1 and FLT 3/ITD mutations had normal karyotyping, elevated TLC, blasts and LDH. In conclusion, NPM1/FLT3-ITD mutations are more common in AML than ALL. In AML, FLT3/ITD mutations were more common than NPM1 mutations. Higher incidence of NPM1 mutations were found in patients with favorable prognosis factors. Patients with poor prognostic factors were more likely to harbor mutations in NPM1/FLT3-ITD. Our data concurs with previously published reports and may reflect similar biology of AML in Indian and western patients.

398: Genetic association of promoter and structural gene variants of Mannan-Binding Lectin (MBL2) gene with susceptibility to vitiligo

[1]**Mitesh Dwivedi**, [2]Kshitij Gupta, [2]Krishana Chaitanya Gulla, [1]Naresh Chandra Laddha, [2]Krishnan Hajela, [1]Rasheedunnisa Begum

[1]Department of Biochemistry, The Maharaja Sayajirao University of Baroda, Faculty of Science, Sayajigunj, Vadodara 390002, Gujarat, India, [2]School of Life Sciences, Devi Ahilya Vishvavidyalaya, Indore, Vigyan Bhavan, Khandwa Road Campus, Indore 452001, Madhya Pradesh, India

Vitiligo is a common depigmenting disorder resulting from the loss of functional melanocytes in the skin and affects 0.5–1% of the world population. In India Gujarat and Rajasthan states have the highest prevalence i.e. ~8.8%. The three prevailing hypotheses for the pathogenesis of vitiligo are the autoimmune, oxidative stress and neurochemical hypotheses. However, none of them explains the entire spectrum of this disorder. Autoimmunity has been suggested to play a major role in the pathogenesis of the disease as is evident by its frequent association with other autoimmune disorders and the presence of

antimelanocyte antibodies in the serum of patients with the disease. The disease does not follow the simple Mendelian inheritance pattern and its mode of heredity suggests that it is a polygenic disease. Mannan-binding lectin (MBL) is a liver-derived calcium dependent serum protein, which plays an important role in innate immune defense. In addition to complement activation via lectin pathway, the protein has several distinct functions including promotion of complement-independent opsono-phagocytosis, modulation of inflammation and promotion of apoptosis. The role of the MBL pathway in complement activation and in the clearance of apoptotic cells suggests that genetic variability in MBL may be involved in the pathogenesis of autoimmune diseases. The aim of this study was to find out whether MBL2 structural and promoter polymorphisms are associated with Gujarat vitiligo patients where the prevalence of vitiligo is alarmingly high. We undertook a case-control study to investigate the association of MBL2 gene exon 1 polymorphisms: codon 52 (rs5030737), codon 54 (rs1800450) and codon 57 (rs1800451) as well as promoter -221 (rs7096206) polymorphism in 90 vitiligo patients and 102 healthy age matched controls. The PCR-heteroduplex analysis was used to investigate these polymorphisms in the MBL2 gene. The genotype and allele frequencies of these MBL2 structural and promoter polymorphisms did not differ significantly between the controls and patient population which shows that there is no association of MBL2 structural and promoter polymorphisms with vitiligo susceptibility (P values: $P < 0.065$ for codon 52, $P < 0.729$ for codon 54, $P < 0.788$ for codon 57 and $P < 0.867$ for -221 promoter polymorphism). In conclusion, our results suggest that the well documented structural and promoter polymorphisms of MBL2 gene may not be associated with vitiligo susceptibility in Gujarat population.

399: Systematic resequencing of the coding exons of the X chromosome in X-linked mental retardation

[1]**Jozef Gecz**, [2]Patrick S. Tarpey, [2]Raffaella Smith, [2]Erin Pleasance, [3]Annabel Whibley, [4]Douglas F. Easton, [5]Michael Partington, [5]Anna Hackett, [5]Michael Field, [6]Roger E. Stevenson, [3]Martin Bobrow, [5]Gillian Turner, [6]Charles E. Schwartz, [3]F. Lucy Raymond, [2]P. Andrew Futreal, [2,7]Michael R. Stratton

[1]Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia, [2]Cambridge Institute of Medical Research, Cambridge CB2 2XY, United Kingdom, [3]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, United Kingdom, [4]Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge CB1 8RN, United Kingdom, [5]GOLD Service, Hunter Genetics, Waratah, NSW 2298, Australia, [6]JC Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC 29646, United States of America, [7]Institute of Cancer Research, Surrey SM2 5NG, United Kingdom

Mental retardation (MR) affects 1–3% of live births and has both genetic and non-genetic causes. A proportion of cases with genetic abnormalities are attributable to mutations of genes on the X chromosome. Although many X-linked MR (XLMR) genes have been reported, identification of more by conventional approaches has become problematic. To address this, we have implemented a strategy in which the coding exons of 719 X chromosome genes have been systematically resequenced for disease-causing variants in 206 XLMR families. This approach has yielded at least 10 new XLMR genes with several more to be validated. Many families still remain to be explained. The study has revealed the pattern of haplotypic coding sequence variation on the X chromosome and indicates that loss of function of $\sim$1% of X chromosome genes is compatible with apparently normal existence. This work has also highlighted issues that may be faced in the future by whole genome screens for rare disease-causing variants in other complex phenotypes.

400: Identification of a novel chromosomal locus in a Belgian FTLD-MND family

[1,2,3]**Ilse Gijselinck**, [1,2,3]Githa Maes, [1,2,3]Julie van der Zee, [1,2,3]Ivy Cuijt, [1,2,3]Karin Peeters, [1,2,3]Maria Mattheijssens, [1,2,3]Christine Van Broeckhoven, [1,2,3]Marc Cruts

[1]Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Universiteitsplein 1, B-2610 Antwerpen, Belgium, [2]Laboratory of Neurogenetics, Institute Born-Bunge, Universiteitsplein 1, B-2610 Antwerpen, Belgium, [3]University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative brain disorder with a prevalence similar to that of Alzheimer's disease in the population below age 65 years. In some patients FTLD symptoms are accompanied by signs of motor neuron disease (MND). A positive family history is observed in up to 50% of FTLD patients indicating a significant contribution of genetics to the etiology of FTLD. A high degree of genetic heterogeneity has been observed with different mutations in the genes encoding the microtubule-associated protein (MAPT), progranulin (PGRN), charged multivesicular body protein 2B (CHMP2B) and valosin containing protein (VCP). Further, two loci on chromosome 9 at 9q21-q22 and 9p13-21 were implicated in FTLD with MND (FTLD-MND). In Belgian familial FTLD patients 65% remained unexplained by mutations in known FTLD genes. Of one patient diagnosed with FTLD-MND, we collected DNA of relatives and performed a genome-wide scan. We identified and finemapped a novel chromosomal locus that was not previously linked to FTLD and/or MND. Haplotype analysis identified a risk haplotype of 23cM that co-segregated with disease. Further reduction of the candidate region and mutation analyses of positional and functional candidate genes are ongoing. Identification of the mutation in the underlying disease gene will significantly contribute to the understanding of neurodegenerative disease mechanisms in FTLD.

401: Identification of tuberous sclerosis causing gene and finding the treatment for the disease

B. R. Gireesha, M. Mahesh, Bagchi Preenon, P. C. Anamika

Best Biotek Research Labs (P) Ltd, #11, 5th cross, Byregowda Layout, Mallathalli, Jnanabharathi Post, Bangalore 56, India

The following work is a Computer Aided Drug Designing (CADD) work on Bioinformatics plateform developed using Computational tools. Tuberous sclerosis (TSC) is a rare genetic disease that causes benign tumors to grow in the brain and on other vital organs such as the kidneys, heart, eyes, lungs, and skin. It commonly affects the central nervous system. TSC is caused by defects, or mutations, on two genes—TSC1 and TSC2. Only one of the genes needs to be affected for TSC to be present. The TSC1 gene, discovered in 1997, is on chromosome 9 and produces a protein called hamartin. Although some individuals inherit the disorder from a parent with TSC, most cases occur as sporadic cases due to new, spontaneous mutations in TSC1 or TSC2. In this situation, neither parent has the disorder or the faulty gene(s). Instead, a faulty gene first occurs in the affected individual. In familial cases, TSC is an autosomal dominant disorder, which means that the disorder can be transmitted directly from parent to child. Homo

sapiens hamartin (TSC1) HGNC: 12362 Human Gene Nomenclature Database [AF013168 (from NCBI database)] was taken for our work. Homology modeling studies were done using template 1G8XA (template information obtained from hhpred server corresponding to IPR007483) and 3D structure of TSC1 protein was modelled. Baikal or Chinese scullcap (*Scutellaria baicalensis*) is commonly used in Chinese herbalism, where it is considered to be one of the 50 fundamental herbs and is used primarily in treating "hot and damp" conditions such as dysentery and diarrhoea. It has been used medicinally for over 2,000 years and recent research has found that the roots contain flavonoids that greatly enhance liver function and also have anti-inflammatory and antiallergenic effects. Wogonin (5,7-dihydroxy-8-methoxyflavone) is a flavonoid derived from the root of *Scutellaria baicalensis*. Based on the known anti-inflammatory and antioxidant activity of wogonin which makes it applicable in the treatment of neurological disorders, hence applicable in the treatment of tuberous sclerosis. The structure of wogonin was made using the software ACD/ChemSketch and saved as MDL*mol file (wogonin.mol). Again, wogonin.mol was opened with Argus Lab and saved as wogonin.pdb which now acts as the ligand (drug) for the receptor TSC1 protein. The receptor TSC1 protein and the ligand wogonin.pdb (drug) were docked by submitting the receptor and the ligand to PATCHDOCK Server.

402: The secondary use of samples. Lessons from the Italian Paediatric Diabetes Studies

[1]**Cecilia Guastadisegni**, [1]Virgilia Toccaceli, [1]Antonia Stazi, [2]Dario Iafusco

[1]Istituto Superiore di Sanita', Viale Regina Elena 299, 00161 Rome, Italy, [2]Second University of Naples, Via Sant'Andrea delle Dame 4, 80138 Naples, Italy

In response to the public's fear of abuse and misuse of genetic information, public policy applied specific regulations to the field of genetics. An authorization for Processing of Genetic Data was issued by the Italian Privacy Authority in 2007. Regulating genetic data separately from general medical data would create major practical problems in health management for it would be hard to differentiate clearly between what is 'medical' and what is 'genetic'. New genetic discoveries have shed light on patient's clinical beneficial reasons. In children diagnosed with diabetes in the first 6 months is now available a genetic test which identifies mutations of the KCNJ11 gene encoding for the pancreatic beta cell potassium channel. This genetic causal mechanism has important therapeutic implications as many children can replace insulin injections with sulfonylurea tablets, highly improving their quality of life. Biological samples of Italian children collected formerly, for a study plan to evaluate the HLA aplotype, were re-used to identify the KCNJ11 mutations to consider the therapeutic switching (before the comprehensive Italian Privacy law in 2003). More than half of patients substituted insulin with oral agents after a variable period from the initial diagnosis (3 months–26 years). Another example is the maturity onset diabetes of the young in which many children are misdiagnosed with type 1 diabetes and treated with insulin while glucokinase mutations need no treatment and HNF-1alpha mutations need sulphonylurea tablets. The re-use of DNA collected for different aims could produce benefits on the quality of life of these patients. Secondary use of identified or coded biological samples, beyond the scope of a specified study, should be permitted in retrospective studies when beneficial therapeutic changes can be achieved. In type 2 diabetes of adolescents concomitant effects of many gene variants, such as the very promising risky variant TCF7L2 and environment contribute to the onset of the disease. The re-use of previously collected samples for polygenic disorders susceptibility tests need a different ethical approach as concordance between population susceptibility associations and individual tests has been poor. The re-consent waiving in the paediatric genetic tests can be justified in the context of reciprocity and accountability between research subjects and research team as the first informed consent creates a relationship and a sense of sympathy with the fate of the children.

403: Association of mannose binding lectin gene variant with leprosy in an Indian cohort

Kshitij Gupta, Krishana Chaitanya Gulla, Krishnan Hajela

School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Takshashila Campus, Devi Ahilya Vishwavidyalaya, Khandwa Road, Indore, India

Mannose-binding lectin (MBL) is an important component of innate immunity, which acts as a pattern recognition molecule of a wide range of infectious agents. On recognition MBL triggers the lectin pathway of complement, leading to the generation of multiple opsonic C3b fragments, resulting in organism uptake by phagocytes, largely by CR1(CD35) receptors. Polymorphisms in exon 1 and promoter of the MBL2 gene have been shown to have a significant effect on the circulating levels of the protein, identified as the cause of the most common immunodeficiency predisposing to infections and autoimmune diseases. The fact that MBL promotes complement activation may be advantageous to intracellular pathogen (e.g. *M. tuberculosis*, *M. leprae*, *L. chagasi*) since they use C3 opsonization and C3 receptors to allow entry of host monocytes/macrophages. However, so far an epidemiological correlation of MBL in leprae patients in India, where the prevalence of leprosy is considerably high, is not established. In the present study we thus, aimed to investigate the association of MBL2 gene exon 1 polymorphisms: codon 52(rs5030737), codon 54(rs1800450) and codon 57(rs1800451) as well as promoter -221 (rs7096206) polymorphism and serum concentration in leprosy patient ($n = 110$) in comparison with controls ($n = 95$) from India. The PCR-heteroduplex analysis was used to investigate these polymorphisms in MBL2 gene. The concentration of serum MBL was determined using commercially available double antibody ELISA. The distribution of MBL2 gene promoter -221(X/Y; rs7096206) polymorphism in patients was significantly different from that of controls with a increased frequency of haplotypes/genotypes associated with low expression of circulating MBL in lepromatous patients when compared with healthy controls. The genotype XY was associated with leprosy [OR 1.39 (95% CI 0.907–2.156) $p = 0.023$]. Difference was not observed in exon 1 polymorphisms in patients and controls. Our results suggest that MBL2 gene polymorphism plays a role in susceptibility to leprosy in Indian patients. There was a significant difference in MBL levels between patients and control. Surprisingly, we found that the concentration of MBL was significantly increased (median controls 354.59 μg/ml, median leprosy patients 603.19 μg/ml; $p = 0.009$ Mann Whitney U test). Since, MBL shows an acute phase response, probably the expression of MBL is increased after chronic infection in patients in spite of higher incidence of low producing genotype. This work was supported by UGC grant No. 31-285/2005SR.

404: Repetitive elements flank both breakpoints of a chromosome 3 inversion in a 3-generation family with short stature

[1]**Ingo Hansmann**, [1,2]Usha Dutta, [1]Dietmer Schlote

[1]Institut für Humangenetik und Medizinische Biologie, Magdeburgerstr. 2, D-06097 Halle (Saale), Germany, [2]Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, India

Chromosomal rearrangements are often associated with a specific phenotype and they are a significant cause of human disorders. Cytogenetic mapping is a powerful tool for identification of such disease genes. Here we report a case of short stature in a girl with a karyotype of 46, XX,inv(3)(p24.1q26.1). Cytogenetic analysis had revealed a familial pericentric inversion 3, being heterozygous in the proband, her mother and grandmother. In order to characterize the breakpoint physically, FISH (fluorescence in situ hybridization) analysis with large YAC (yeast artificial chromosome) and BAC (bacterial artificial chromosome) clones were performed. Four p specific YACs and six BAC clones were used as probes for FISH. YAC clone CEPHy904H0787 (1090 kb) gave a split signal on the metaphase chromosomes of the proband. The split signal indicates that the target sequence carries the inversion breakpoint. Further, two BAC clones RP11666G20 and CTD2007B5 were identified spanning the breakpoint region, assigning the breakpoint to 3p24.1 and thus narrowed down the breakpoint region to 97.5 kb. Out of the 15 YACs and 10 BACs selected on the q arm, YAC CEPHy904G07889 (1610 kb) and BAC clone RP11-12N13 showed a split signal assigning the breakpoint to chromosomal band 3q26.1. Using sub cloned fragments of these BACs as well as Long range PCR products as probes the breakpoints were now located within a region of 3 and 5.3 kb on p and q respectively. Analysis of the genomic sequence surrounding the inversion breakpoints revealed 30% repetitive nature of the DNA containing LTR33A, MER67C, L2 and MER67D elements on p region and LTR16C, MER20, MLT2B1, LTR1B, simple repeats and low copy repeats on q region. We determine that the breakpoints occurred between these repetitive regions. The presence of these repetitive elements, especially MER and LTR elements at the junction of the breakpoints suggest that the inversion may be the result of these repetitive elements. Our findings will help to provide a better understanding of the molecular mechanism underlining the spontaneous chromosome rearrangements in the human genome.

405: Genotype: molecular phenotype correlation using lymphoblastoid cell lines from patients with multiple primary neoplasia

Tabish Hussain, Ashwin Kotnis, Rajiv Sarin, Rita Mulherkar

Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai 410210, India

Tobacco related cancer (TRC) accounts for almost half the global burden of cancer and arises from a complex gene-environment interaction. While prolonged exposure to tobacco with or without other carcinogens plays a central role in the genesis of these cancers, various host genetic factors could significantly modulate the risk of developing tobacco related cancer. One of the major TRCs with high morbidity in Indian men is upper aero digestive tract (UADT) cancer. Despite the impressive advances that have been made in molecular and cell biology to cure or control cancers, annual risk of UADT remains constant or increases due to threats posed by generation of second primary neoplasm, giving rise to multiple primary neoplasias (MPN). We hypothesize that the response to genotoxic drugs varies in different individuals depending on their genetic make-up. In the present study, LCLs were prepared from MPN patients and controls since they serve as an infinite source for carrying out various studies. Theses LCLs were used as an in vitro model to study the effect of genotoxins. We have compared apoptosis and gene expression profiles between UADT and MPN patients with tobacco habit and matched healthy controls, in response to DNA damaging agents. The apoptosis assay was done by flow cytometry after exposing the cells to two genotoxic agents such as gamma radiation and benzo[a]pyrene diol epoxide (BPDE, a tobacco specific carcinogen). MPN patients showed less apoptosis as compared to controls which could be attributed to defective DNA repair in MPN patients.

We have carried out microarray experiments using 19K cDNA arrays from Toronto Microarray Centre, after exposing LCLs to genotoxic agents. Some of the candidate, differentially expressed genes are being studied. The results obtained will be compared in patients and controls with respect to the genetic variations in their DNA repair genes. The information from these experiments can then be translated to establish the correlations between single nucleotide polymorphisms (SNPs) and complex diseases and to finally draw a genotype-molecular phenotype correlation.

406: Significance of TP53 codon 72 polymorphism in breast and lung cancer showing different xenobiotic potential spectrum

[1]**Rakhshan Ihsan**, [1]M. Kaushal, [1]A. K. Mishra, [2]J. Sharma, [3]D. Behera, [3]A. Jaiswal, [3]K. Gupta, [4]E. Zomawia, [1]Sujala Kapur, [1]Sunita Saxena

[1]Institute of Pathology (ICMR), Safdurjung Hospital Campus, New Delhi 110029, India, [2]Dr. B. Borooah Cancer Institute, Guwahati, Assam, India, [3]L.R.S. Institute of Tuberculosis and Allied Diseases, Aurobindo Marg, New Delhi, India, [4]Civil Hospital, Aizwal, India

Introduction: Genetic polymorphisms at the genes involved in tumorigenesis may determine individual susceptibility of cancer. Germline TP53 mutations have been reported to be associated with inherited cancer risk and codon 72 polymorphic variants have also been studied as potential susceptible genotypes. Significant association between the codon 72 polymorphism and risk of cancer have been reported, although the results with regard to most cancer diseases, including breast and lung carcinomas remain inconclusive.
Hypothesis: The Arg72 variant demonstrates apoptotic ability while the proline variant has enhanced DNA repair capacity showing increased growth arrest. DNA repair function can be imposed by DNA damage caused through DNA adducts formed due to absence or impaired xenobiotic metabolizing enzymes. Thus, TP53 codon 72 polymorphism along with genes modulating the levels of xenobiotics in human body might significantly contribute to the incidences of these cancers.
Materials and methods: A case control study including 130 breast cancer cases, 120 lung cancer cases and 250 age matched healthy controls was performed. PCR-RFLP analysis and multiplex PCR technique was conducted to identify TP53 codon 72 genotypes and GSTM1 and GSTT1 gene polymorphism respectively.
Result: The frequency of three TP53 genotypes Arg/Arg, Arg/Pro and Pro/Pro found in breast cancer were 24.3, 42.3, and 33.3%, respectively and in lung cancer 22.2, 48.24 and 29.38% respectively. The lung and breast cancer patient with Pro/Pro genotype showed an increase risk (OR = 1.3846; CI = 0.834–2.2987) and significantly increased OR (1.882; CI = 1.169–3.0299; p = 0.008) respectively, with other genotype, in comparison to controls. Absence of both GSTM1 and GSTT1 genes confers 23 and 57% less chance of developing lung cancer, (OR = 0.7745, CI = 0.473–1.2681; OR = 0.4375, CI = 0.2375–0.805 respectively).
Discussion: The inheritance of a homozygous Pro/Pro genotype of TP53 increased the risk of breast cancer to about more than 1.8 times (OR = 2.571; 95% CI = 1.453–4.550). The preliminary study shows a probable interactive association between the genes and the studied cancers.

407: Comprehensive characterization of genetic aberrations in T-lymphomas in children

[1]**D. Ilencikova**, [1]K. Hlinkova, [2]J. Suvada, [3]L. Plank, [4]E. Bubanska, [5]I. Jenco

[1]National Cancer Institute, Department of Cancer Genetics, Klenova 1, 833 10 Bratislava, Slovakia, [2]University Children's Hospital, Department of Paediatric Oncology, Limbova 1, 833 40 Bratislava, Slovakia, [3]Jessenius Medical Faculty of Comenius University, Department of Pathology, Kollarova 2, 036 59 Martin, Slovakia, [4]University Hospital, Clinic of Children Oncology, Banska Bystrica, Nam. L. Svobodu 1, 975 17 Banska Bystrica, Slovakia, [5]University Hospital, Department of Children Oncology, Tr. SNP 1, 041 90 Kosice, Slovakia

Purpose: T-lymphomas in children are relatively rare—approximately 12% of NHL. T-NHL present a heterogenous group of malignant diseases with different clinical features and responses to the chemotherapy. Genetic examinations of T-lymphomas in children can specify diagnosis of disease and supply information about progression of malignance.
Method: In 1999–2008, 26 patients were diagnosed and examined at our Department of Cancer Genetics. DNA isolated from bone marrow, peripheral blood, fixated and native tissues of lymph-nodes was used as the material for investigation. We screened these samples for frequent chromosomal aberrations and imbalances by cytogenetic methods, FISH analyses, heteroduplex analyses, CGH and array-CGH. The heteroduplex analyses were used to find the specific rearrangement of genes for T-cellular receptors. FISH analysis detected chromosomal aberrations via these types of probes: sil/tal (del(1)(p32,p32)), TLX3(t(5,14)(q35,q32)), TCRα/δ(14q11.2), TCRβ (7q34), TCRγ(7p14), p16(9p21), 9q34/cep9. Five tumors were screened for chromosomal imbalances by comparative genomic hybridization (CGH).
Results: Genomic aberrations were detected in 78% of T-lymphomas. Rearrangements of TCRα/δ, TCRβ and TCRγ were found in 74, 37.7 and 60% of T-lymphomas, respectively. Recurrent gains were identified on 7q34 (20%), 7p14 (10%), and 9q34 (10%). Recurrent losses were found on chromosomes 9q34 (20%) and p16 (10%). In one case, combined gains on 7q34, 7p14 and 9q34 were detected. Also in one case, combined deletions on 14q11, 7q34, 9q34 and 9p21 were identified. Some of the array-CGH in tumor tissue sections were verified by iFISH.
Conclusion: We present genetic results in correlation with survival of children with T lymphoma. We evaluated prognostic significance of detected chromosomal aberrations. This work was supported by MH SR 2005/16-NOU-01.

408: Evidence of CHEK1 and EI24 as candidate tumor suppressor genes associated with the development of uterine cervical carcinoma

[1]**Dipanjana Indra**, [1]Ratnesh K. Singh, [1]Sraboni Mitra, [1]Ranajit K. Mondal, [1]Partha S. Basu, [2]Anup Roy, [3]Susanta Roychoudhury, [1]Chinmay K. Panda

[1]Chittaranjan National Cancer Institute, 37 S.P. Mukherjee Road, Kolkata 26, India, [2]Calcutta Medical College and Hospital, 88 College Street, Kolkata 73, India, [3]Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata 32, India

Microcell hybrid study suggests the presence of at least one tumor suppressor gene (TSG) in chromosome 11 associated with the development of uterine cervical carcinoma (CACX). The chromosomal 11q23-24 region is known to be deleted in a wide variety of human epithelial malignancies, including CACX. Our previous study has revealed chromosomal 11q23.3-24.3 region to be highly deleted in CACX. In this study, high-resolution deletion mapping of the chromosomal 11q23.3-24.3 region (7.8 Mb) was done with ten microsatellite markers in nineteen cervical intraepithelial neoplasia (CIN), ninety-six primary CACX and two CACX cell lines. Deletion mapping identified three discrete regions with high frequency of deletions, viz. 11q23.3 (D1), 11q24.1-24.2 (D2) and 11q24.2 (D3). Deletions in D1, D2 and D3 regions progress gradually with tumor progression. The D1 region seems to be associated with CIN and deletions in D2 and D3 regions increased significantly with progression of tumor ($P = 0.01$). To date no candidate TSGs have been mapped to D1 region. The D2 region harbors the candidate TSG LOH11CR2A (BCSC1). The D3 region harbors the two candidate TSGs CHEK1 and EI24/PIG8. CHEK1 promoter methylation was seen in 31% samples and significantly increased with tumor progression ($P = 0.04$) in comparison to only 5% promoter methylation of EI24/PIG8. However, expression analysis of the candidate TSGs CHEK1 and EI24/PIG8 revealed significant reduction in tumors in comparison to normal cervical samples ($P = 0.04$), the extent of reduction being 54 and 62% respectively. Thus, it indicates that CHEK1 and EI24 are candidate TSGs associated with the development of CACX.

409: Intercellular genomic variations manifesting as aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain

[1,2]**I. Y. Iourov**, [1,2]S. G. Vorsanova, [3]T. Liehr, [1,2]Y. B. Yurov

[1]National Research Center of Mental Health, RAMS, Zagorodnoe 2, Moscow, Russia, [2]Institute of Pediatrics and Children Surgery, Rosmedtechnologii, Taldomskaya 2, Moscow, Russia, [3]Institute of Human Genetics and Anthropology, D-07740, Jena, Germany

The human brain has been recently suggested to possess aneuploid cells, but the nature and magnitude of aneuploidy in the normal and diseased brain remain obscure. Here, we have monitored chromosome variation (loss/gains of whole chromosome or aneuploidy) in the cerebral cortex of the normal, Alzheimer's disease (AD) and ataxia telangiectasia (AT) brain by molecular cytogenetic approaches scoring more than 420,000 neural cells. We have determined the mean rate of stochastic aneuploidy in the normal human brain as 0.5% per chromosome (95% CI 0.2–0.7%; SD 0.2%). The overall proportion of aneuploid cells in the normal brain was approximately 10%. In the AD brain, the level of stochastic aneuploidy was not significantly increased comparing with sex- and age-matched controls. However, dramatic 10- to 15-fold increase of chromosome 21-specific aneuploidy (hypoploidy and hyperploidy) was detected in AD cerebral cortex (6–15% versus 0.8–1.8% in control). In the AT brain, we observed a two to fivefold increase of stochastic aneuploidy randomly affecting different chromosomes (mean 2.1%; 95% CI 1.5–2.6%; SD 0.8%). The overall proportion of aneuploid cells in the brain of AT individuals ranged between 20 and 50%. Our data indicate mosaic neural aneuploidy to be a newly identified feature of early and late onset neurodegenerative diseases similarly to chromosome syndromes and cancers hallmarked by aneuploidy. We conclude that neural aneuploidy differentially contributes to intercellular genomic variation in the normal, AD and AT brain. Together, this suggests aneuploidization to be a likely mechanism for altered cellular physiology mediating the pathogenesis of neurodegenerative diseases. Supported by ATCP USA.

410: Genome wide structural variations in malignant glioma samples of Indian population

[1]**Prerana Jha**, [2]Pankaj Jha, [1]Pankaj Pathak, [2]Vinod Scaria, [3]Kunzang Chosdol, [3]Subrata Sinha, [2]Mitali Mukerji, [1]Chitra Sarkar

[1]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India, [2]Functional Genomics Unit, Institute of Genomics and Integrative Biology, CSIR, Mall Road, Delhi, India, [3]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India

Gliomas are most common primary brain tumors and include astrocytic, oligodendroglial and mixed oligoastrocytic tumors of various histological grades of malignancy. Recently, role of molecular genetics in prognostication of these tumors has been recognized. Thus primary glioblastomas (GBMs) are characterized by epidermal growth factor receptor (EGFR) amplification while secondary GBMs show p53 mutation. Hallmark of oligodendrogliomas is loss of heterozygosity (LOH) of 1p and 19q which is associated with good prognosis and response to chemotherapy. Occasional cases of GBM with 1p/19q loss have been reported and a new entity namely GBM with oligo component (GBMO) has been introduced. However the prognostic significance in GBMO remains controversial because very few studies are available. Hence this study was undertaken to evaluate and compare 1p/19q status in gliomas of different types and grades along with other molecular changes viz. p53 immunoreactivity and EGFR amplification. The study was complemented by molecular karyotyping using Infinium CNV370 chip for high-density single nucleotide polymorphism (SNP) array. Fourty nine gliomas classified by WHO classification (2007) were analyzed for 1p/19q LOH by microsatellite PCR, p53 by immunohistochemistry, EGFR amplification by FISH and RT-PCR. About 70% of oligodendrogliomas and oligoastrocytomas showed combined 1p and 19q LOH. No astrocytic tumors of grade II and III showed any deletion of 1p/19q. In contrast to oligos, GBMs were an interesting group with very few microsatellite marker of 1p/19q loss, 1p-LOH was detected in 23%, 19q-LOH in 30% and combined 1p and 19q in 6.7% cases. p53 immunoreactivity was found in 40% GBMs and EGFR amplification was 25% unlike 40–50% reported in western literature. Both of these changes were absent in oligodendrogliomas. Genome wide structural variation analysis was done on contrasting cases, e.g. GBMO vs conventional GBM, besides other GBMs and oligos. Interestingly, GBMO had complete 1p and 4q LOH, while conventional GBM had partial LOH. It is generally known from literature that a total loss of 1p in oligodendrogliomas have best prognosis, while a segmental loss in GBMs show a poor outcome. It is clinically important to differentiate these histological subtypes of gliomas because they carry different prognostic significance. It also raises a question as to whether the GBMO subset with complete loss of 1p should be classified as anaplastic oligoastro Grade-IV.

411: Molecular markers of human brain tumors and their participation in cellular signaling pathways

[1]**Vadym Kavsan**, [1]Kateryna Shostak, [1]Vladimir Dmitrenko, [1]Oxana Boyko, [1,2]Andrei Marusyk, [3]Yuri Zozulya

[1]Institute of Molecular Biology and Genetics, 150 Zabolotnogo Str., Kiev, Ukraine, [2]University of Colorado Health Sciences Center, Denver, Colorado, United States of America, [3]A. P. Romodanov Institute of Neurosurgery, 32 Manuilsky Str., Kiev, Ukraine

The aim of this investigation is identification and characterization of genes with significant changed expression in brain tumors and their possible interaction with signaling pathways. Such knowledge is necessary not only for understanding tumorigenesis, but also normal brain functioning. SAGE, northern, RT-PCR, western blot analysis, histochemistry were used to identify 129 genes with fivefold changes of expression in glioblastomas, the most aggressive form of human brain tumors. This altered pattern of gene expression in tumor cells can be viewed as a molecular marker in the analysis of malignant progression of astrocytic tumors, and as possible clues for the mechanism of disease. Moreover, several genes overexpressed in glioblastomas produce extracellular and membrane proteins or proteins involved in signaling pathways, thereby providing possible therapeutic targets. Next step includes functional analysis of encoded proteins, their potential partners and participation in cellular signaling pathways. High levels of CHI3L1 gene expression, the product of which (HC gp-39) reveals a mitogenic effect, similar to the effect of insulin-like growth factor I (IGF-I), correlates with unfavorable course of the disease. Since deregulation of the IGF system/HC-gp39 is a frequent pattern in tumours, IGFs/IGFBPs/HC-gp39 should be included in the panel of tumour markers used for the diagnosis and serological surveillance in various malignancies. As a functional antagonist to the potential oncogene CHI3L1, gene TSC22D1 (coding for TSC-22) has significantly lower expression in astrocytic gliomas. Differential expression of TSC22D1 was confirmed by histochemical analysis. TSC-22 may serve as a mediator of TGF-β signals. A substantial decrease of TSC22D1 expression on the RNA and protein levels revealed in glial tumors together with the known negative role of TSC-22 in cell proliferation regulation are evidence about its tumor-suppressed function. It allows to offer TSC-22 as a prognostic factor for gliomas. Further characterization of these genes will thus allow them to be exploited in molecular classification of glial tumors, diagnosis, prognosis, and anticancer therapy. Novel antisense and iRNA strategies targeting components of cellular signaling pathways may offer additional options for treatment of malignant gliomas.

412: Role of *CaMKIV* and *PRM* genes in idiopathic male infertility

[1]**Arun Khattri**, [2]Nalini Gupta, [2]Baidyanath Chakravarty, [3]Mamta Deenadayal, [1]Lalji Singh, [1]Thangaraj Kumarasamy

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India, [2]Institute of Reproductive Medicine, Salt Lake, Kolkata, India, [3]Infertility Institute and Research Centre, Hyderabad, India

During spermiogenesis chromatin of the sperm becomes highly compact due to the replacement of histones by protamines (PRM) and improper replacement can cause sperm DNA damage leading to male infertility. Calmodulin dependent protein kinase 4 (CAMK4) phosphorylates the protamines, which is responsible for interaction of protamines with chromatin. Therefore, we screened *CAMK4* and *PRM1, 2 & 3* genes, which are involved in the above functions, in Indian men which includes 278 infertile and 249 fertile men. We have observed a total of 55 polymorphic sites in these genes. Although the frequency of many SNPs was low, 9 SNPs in *CAMK4* gene and 15 SNPs *PRM* genes were observed exclusively in infertile men and 2 SNPs, g.-192_193insC in *CAMK4* and g.-25:C>T in *PRM3* showed significant difference between infertile and fertile men. The g.-25:C>T substitution is present in the upstream of the *PRM3* gene and may be affecting the expression of *PRM3*, gene. This novel predicted *PRM3* gene was confirmed for its transcription and full-length transcript was isolated and found to have testis specific expression.

We further predicted that many infertile men-specific mutations could cause either loss or appearance of potential binding sites for various splicing factors. The SNPs of *PRM1* and *PRM2* genes were found to be in LD, but not in *PRM3*. Haplotype analysis revealed that the haplotype PRM-H6 conferred significant risk for infertility. We addressed for first time the association SNPs and haplotypes of

CaMK4 and *PRM* genes with male infertility in Indian men. Further analysis of these genes on other populations is required to validate them as a candidate for infertility in males.

413: Profiling of esophageal squamous cell carcinoma using whole genome mRNA and aCGH arrays

[1]**Charles Jacob Harrys Kishore**, [1]Manoj Kumar Kashyap, [1]Arivusudar Marimuthu, [1]Shivakumar Keerthikumar, [1]T. S. Keshava Prasad, [2]Riaz Mahmood, [3]Sudha Rao, [4]M. Vijayakumar, [4]K. V. Veerendra Kumar, [4]Rekha V. Kumar, [1,5]Akhilesh Pandey

[1]Institute of Bioinformatics, Bangalore 560066, India, [2]Kuvempu University, Shimoga 577451, India, [3]Genotypic Technologies, Bangalore 560001, India, [4]Kidwai Memorial Institute of Oncology, Bangalore 560029, India, [5]The Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States of America

Esophageal squamous cell carcinoma (ESCC) is a common cancer worldwide and has a dismal survival rate partly due to the lack of modalities to identify the cancer at an early stage. Molecular mechanisms contributing to initiation and progression of esophageal squamous cell carcinoma have not been well investigated. Accumulation of mutations and alterations in the expression of various genes and chromosomal aberrations result in the carcinogenesis of the esophagus. The development of microarray technologies have enabled the identification of gene expression and genomic alterations. In order to identify molecular targets for diagnosis and possibly treatment, we have analyzed the genome-wide gene expression profiles of 20 surgical specimens of ESCC and compared them to their adjacent normal epithelium using gene expression arrays. Comparative Genomic Hybridization array (aCGH) experiments were also done on the same samples to detect corresponding chromosomal copy number changes. From these microarray studies we have identified several candidate genes to be significantly upregulated, that have not been reported earlier. This list includes Oral Cancer Overexpressed 2 (TMEM16A), fibroblast activation protein (FAP), Periostin (POSTN), Versican (VCAN) and Lumican (LUM). We have identified a locus 11q13.3 to be overamplified in 50% of the cases. Among the amplicons identified in the region, TMEM16A located at 11q13.3 shows an amplification in 80% of the samples investigated. This might be responsible for the transcriptional upregulation of TMEM16A at the mRNA level. Two novel overexpressed genes (TMEM16A and FAP) were validated at the protein level by immunohistochemical staining of the tissue samples using commercially available tissue microarrays (TMAs). Overexpression of TMEM16A was observed in 116/118 (98%) cases, while FAP overexpression was in 79/116 cases (68%) cases. Osteopontin that has previously reported in earlier studies was observed in 114/118 (97%) cases. These data highlight genes and genomic regions that contribute to esophageal squamous cell carcinoma and may be used as potential therapeutic targets.

414: Apparently balanced, de novo complex chromosome rearrangement: 46, XY, t(8;10;12) associated with Autism: cytogenetic and molecular studies

[1]**D. S. Krishna Murthy**, [2]Sabita K. Murthy, [3]K. S. Sperling, [4]H. Tönnies

[1]Microtest Innovations, G 05 Discoverer, IBAB, ITPB, Bangalore, India, [2]Medical Genetics Center, Al Wasl Hospital, DOHMS, Dubai, United Arab Emirates, [3]Institut für Humangenetik, University of Berlin, Berlin, Germany, [4]Institut für HumangenetikGenetische Beratungsstelle Berlin, Chromosomendiagnostik, Molekulare Zytogenetik, Augustenburger, Germany

Autism spectrum disorder (ASD) is a rare behavioral phenotype defined by a qualitative impairment in reciprocal social interaction, impairment in communication and imaginative activity, and a markedly restricted repertoire of activities and interests. It is the most severe form of pervasive developmental disorders of childhood. Using even strict diagnostic criteria, the currently described etiologies of autism are heterogeneous, with the majority of cases continuing to be idiopathic. At present, it is not clear whether autism is merely a behaviorally defined phenotype arising from diverse etiologies or a separate category of psychological dysfunction for which some unifying etiology exists. Complex chromosome rearrangements (CCR) are rare structural abnormalities involving at least three chromosomes and three or more break-points. We report here an 8-year-old boy with ASD and a CCR involving chromosomes 8, 10 and 12. His Karyotype showed—46,XY, der(8),t(8;12)(q22;q22), der(10), t(8;10)(q22;q25), del(12)(q22->qter). Family history was non-informative suggesting a possible de novo rearrangement. The karyotype in both the parents confirmed normal. This case suggests that aberrations at 8q22, 10q25, and/or 12q22 may result in pervasive developmental disorder, associated with mild cognitive delay. We discuss the possible relationship of this chromosome abnormality to the etiology of his autism.

415: Genomic disorders: a new class in the taxonomy of human disease

Dhavendra Kumar

Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom

Genetic diseases are recognized as one of the major categories of human disease. Traditionally genetic diseases are subdivided into chromosomal (numerical or structural aberrations), monogenic or Mendelian diseases, multifactorial/polygenic complex diseases and mitochondrial genetic disorders. With the advent of newer molecular techniques, a number of new disorders and dysmorphic conditions are delineated in detail. These conditions do not conform to the conventional inheritance patterns and mechanisms are often complex and unique. Examples include submicroscopic microdeletions and microduplications, triplet repeat disorders, epigenetic disorders due to imprinting, defective transcription or translation due to abnormal RNA patterning and pathogenic association with single nucleotide polymorphisms (SNPs) and copy number variations (CNVs). Among these several apparently monogenic disorders result from non-allelic homologous recombination (NAHR) associated with the presence of low copy number repeats (LCNRs) on either side of the critical locus or region. The term disorders of 'genome architecture' has also been used for these disorders. Examples include Charcot-Marie-Tooth disease type 1A (CMT1A), Smith-Magenis syndrome, neurofibromatosis type 1 deletion and many more that appear on genetic databases with an assigned OMIM number. Clinical heterogeneity and molecular mechanisms in some of the major disorders of genome architecture are presented for critical appraisal.

416: Centric fission of Chromosome 5 in a couple with recurrent spontaneous abortions

[1]**M .Kumar**, [1]R. Dada, [1]M. Tanwar, [1]D. Pathak, [2]N. Malhotra, [2]S. Mittal

[1]Lab for Molecular Reproduction and Genetics, 1027A, Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar,

New Delhi 110029, India, [2]Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

Centric fission is a rarely reported chromosomal abnormality in humans. Centric fission results when metacentric or submetacentric chromosome splits at the centromere giving rise to two stable telomeric products. We report a centric fission of chromosome 5 in a healthy female suffering from recurrent miscarriages. A couple was referred for cytogenetic analysis with bad obstetric history. Pregnancy history, age, occupation, disease information and all other medical records were reviewed. The wife had history of three abortions. The etiology of recurrent abortions is often unclear and may be multifactorial. Couples having multiple miscarriages are at risk for carrying chromosomal abnormalities. Majority of the studies have shown that most of the chromosomal abnormalities associated with recurrent abortions are numerical and structural which may adversely affect embryonic development but there are reports of recurrent miscarriages due to centric fission of the chromosomes. Peripheral blood was collected and lymphocyte cultures were set up for chromosomal analysis. Atleast 20 metaphases were analysed using GTG banding. The husband was cytogenetically normal and wife had 46,XX,-5,+fis(5)(p10),+ fis(5)(q10) chromosomal complement. Both fission products were mitotically stable. To the best of our knowledge this is the first report on centric fission of chromosome 5 as the individuals with stable centric fission products have rarely been documented in humans. In this case, the centric fission does not cause any significant dysmorphic features and mental handicap. But heterozygous individuals for centric fission appear to be at increased risk for production of unbalanced gametes which consequently increased risks for spontaneous abortion, stillbirth and live born infants. However, even between homologous chromosomes, the size of centromeres is different from each other. A further study including more such cases is required to understand the etiology of recurrent spontaneous abortions in detail.

417: Cytogenetic analysis in myelodysplastic syndrome

[1]**Rakesh Kumar**, [2]Rekha Chuabey, [1]Dhananjay Pathak, [2]Renu Saxena, [1]Rima Dada

[1]Lab for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Science, Ansari nagar, New Delhi 110029, India, [2]Department of Hematology, All India Institue of Medical Sciences, Ansari nagar, New Delhi 110029, India

Myelodysplastic Syndrome (MDS) is a clonal disorder of haematopoietic stem cells and results in progressive cytopenia and defect in erythroid, myeloid and megakaryocytic maturation. The diagnosis of MDS is difficult to establish based on morphological features alone because dysplasia is not always be detectable and the presence of dysplasia is not itself evidence of clonal disorder. As a result, the detection of clonal cytogenetic abnormality has a major role in diagnosis and classification of MDS and determining the prognosis. In an attempt to assess the frequency and characteristics type of abnormal clones, cytogenetic analysis was carried in 25 MDS cases. Cytogenetic analysis of bone marrow cells and peripheral blood cells was carried by 24 h unstimulated cell culture and 72 h stimulated blood culture respectively. Chromosomes were obtained by GTG bands. On analysis 12 out of 25 patients had abnormal karyotypes. In eight patients cytogenetic changes were characteristic of those reported for MDS: del(5q)[n = 2], monosomy [n = 3], monosomy 7[n = 2] and one case had Y chromosome loss. Out of 12 cases with cytogenetic abnormalities four cases had cytogenetic abnormalities which have not been previously reported in MDS cases. Out of these four cases: trisomy 9, t(12q-6q), iso(9q) and t(12p-6q) were found in one case each. Long term follow-up and larger studies of such cases are required to determine the malignant potential of these clones and their prognosis.

418: Association of TNF -308 and -238 promoter polymorphisms with vitiligo susceptibility in Gujarat population

Naresh C. Laddha, Mitesh Dwivedi, Amina R. Gani, Krishna J. Patel, Tejas G. Oza, Barkha Jain, Rasheedunnisa Begum

Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India

Genetic polymorphisms in the promoter region of the tumor necrosis factor-α gene (TNF) are involved in the regulation of its expression, and are found to be associated with various autoimmune diseases. We have investigated two polymorphisms in the promoter region of the TNF gene (-308 G/A; rs1800629 and -238 G/A; rs361525) for their role in the susceptibility to vitiligo, by a case–control study involving 120 patients and 153 healthy age-matched controls in Gujarat population where the prevalence of vitiligo is alarmingly high ($\sim$8.8%). Vitiligo is a common dermatological disorder of the epidermis and hair follicles, manifested clinically as expanding hypopigmented lesions of the skin. It affects 0.5–1% of the world population. The contribution of genetic factors in susceptibility to vitiligo is exemplifed by familial clustering. About 20% of vitiligo patients have at least one first-degree relative affected. Cytokines are important mediators of immunity, their response due to imbalance or deficiency in the cytokine network may largely determine disease susceptibility and severity. TNF-α, a multifunctional cytokine, is involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of autoimmune diseases. It is also a paracrine inhibitor of melanocytes. The single nucleotide polymorphisms at positions -308 (G/A) and -238 (G/A) which involve substitution of G for A and resulting in the AA genotype, leads to a higher rate of TNF gene transcription than the wild-type GG genotype. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for the analysis of these promoter polymorphisms. Overall, the distribution of the genotype frequencies and allele frequencies of TNF -308 G/A ($P < 0.013$; $P < 0.0002$ respectively) and -238 G/A ($P < 0.0016$; $P < 0.0001$ respectively) were significantly different between the vitiligo patients and the healthy controls. In conclusion, our results suggest that the well documented promoter polymorphisms of TNF gene may be a genetic risk factor for vitiligo susceptibility in Gujarat population.

419: Mutation analysis of PAH gene and characterization of a recurrent deletion mutation in Korean patients with phenylketonuria

[1]**Yong-Wha Lee**, [2]Dong Hwan Lee, [3]Nam-Doo Kim, [4]Seung-Tae Lee, [5]Jee Young Ahn, [6]Tae-Youn Choi, [1]You Kyoung Lee, [1]Hee Bong Shin, [4]Sun-Hee Kim, [4]Jong-Won Kim, [4]Chang-Seok Ki

[1]Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital, Soonchunhyang University College of Medicine, Bucheon, South Korea, [2]Pediatrics, Soonchunhyang University College of Medicine, Seoul, South Korea, [3]R and D Center, Equispharm Co., Suwon, South Korea, [4]Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University

School of Medicine, Seoul, South Korea, [5]Laboratory Medicine, Soonchunhyang University Gumi Hospital, Gumi, South Korea, [6]Laboratory Medicine, Soonchunhyang University Hospital and Soonchunhyang University College of Medicine, Seoul, South Korea

Phenylketonuria (PKU; MIM 261600) is an autosomal recessive metabolic disorder caused by a deficiency of phenylalanine hydroxylase (PAH; EC 1.14.16.1). Point mutations in the PAH gene are known to cause PKU in various ethnic groups, and large deletions or duplications account for up to 3% of the PAH mutations in some ethnic groups. However, a previous study could not identify ~14% of the mutant alleles by sequence analysis in Korean patients with PKU, which suggests that large deletions or duplication might be frequent causes of PKU in Koreans. To test this hypothesis, we performed multiplex ligation-dependent probe amplification (MLPA) for the identification of uncharacterized mutant alleles after PAH sequence analysis of 33 unrelated Korean patients with PKU. Bi-directional sequencing of the PAH exons and flanking intronic regions revealed 27 different mutations, including four novel mutations (two missense and two deletion mutations), comprising 57/66 (86%) mutant alleles. MLPA identified a large deletion that encompassed exons 5 and 6 in four patients, another large deletion that extended from exon 4 to exon 7 in one patient, and a duplication of exon 4 in one patient. Chromosomal walking characterized the deletion breakpoint of the most common large deletion that involved exons 5 and 6 (c.456_706+138del). The present study shows that the allelic frequency of exon deletion or duplication is 9% (6/66) in Korean PKU patients, which suggests that these mutations may be frequent causes of PKU in Korean subjects.

420: Identification of chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype

[1,2]**Sheroy Minocherhomji**, [1]Arundhati S. Athalye, [1]Prochi F. Madon, [1]Dhananjay Kulkarni, [1]Shonali A. Uttamchandani, [1]Firuza R. Parikh

[1]Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, 15, Dr. G. Deshmukh Marg, Mumbai 400 26, India, [2]Psychiatry Research Program, Jaslok Hospital and Research Centre, 15, Dr. G. Deshmukh Marg, Mumbai 400 26, India

Gene regulation in differentiated cells of multi-cellular organisms is critical for development. Activation/repression of normally repressed/expressed genes could lead to the onset of disease phenotype. Compartmentalization of higher order eukaryotic genomes into silent heterochromatin and active euchromatin represents the degree of gene expression. Chromosomal polymorphic variations, particularly in the heterochromatic region of chromosomes 1, 9, 16, Y and the nucleolar organizing regions of acrocentric chromosomes are known to occur in the general population. We carried out a comparative case-controlled association study using cytogenetic techniques to compare the frequency of chromosomal variations in infertile individuals versus fertile controls. A total of 760 infertile individuals were studied compared to 555 controls. 380 infertile women were divided based on them having bad obstetric history (BOH, Group A), reproductive spontaneous abortions (RSA, Group B) or primary infertility (Group C). The 380 infertile men consisted of men with severe male factor infertility (Group D) or whose female partners had BOH (Group E), RSA (Group F), or primary infertility (Group G). A statistically significant increase in the frequency of total chromosomal variants in infertile women (28.31% vs. 15.16%; P = 0.0007) and infertile men (58.68% vs. 32.55%; P = 0.0002) was observed vs. controls. Of the 380 infertile women studied, Group C showed the highest frequency of 9qh+ (16.22% vs. 6.41%; P = 0.001) compared to controls. Among the 380 men, the frequency of 9qh+ was 14.69% (P = 0.001) in Group D and 16.44% (P = 0.004) in Group G, vs. 4.25% in fertile controls. The frequency of Yqh+ was statistically significant in Group E (30.20%; P = 0.0009) and Group D (29.37%; P = 0.001). Our study shows a highly significant increase in chromosomal variants such as 9qh+ in infertile men and women, and Yqh+ in infertile men vs. controls. We postulate a possible mechanism involving transcriptional activation of nuclear stress bodies in the heterochromatic region of chromosomes being responsible for the association of polymorphic variations with certain clinical conditions, along with the involvement of epigenetic mechanisms of gene regulation and control, histone modification, chromatin rearrangement, and genomic DNA methylation. Further analysis of these heterochromatin regions using immunostaining techniques and higher resolution molecular genetic techniques could improve our understanding of this complex disorder.

421: Studies on interplay among TP53, TP73 and MDM2 loci at the risk of tobacco associated leukoplakia and oral cancer and analysis of apoptotic property of wild type and mutant TP53 gene under different polymorphic background

[1]**Chaitali Misra**, [2]Mousumi Majumder, [1]Swati Bajaj, [2]Sourabh Ghosh, [2]Bidyut Roy, [3]The Indian Genome Variation Consortium, [1]Susanta Roychoudhury

[1]Molecular and Human Genetics Division, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700032, India, [2]Human Genetics Unit, Biological Sciences Division, Indian Statistical Institute, 203, Barrackpore Trunk Road, Kolkata 700108, India, [3]Nodal Laboratory, Institute of Genomics and Integrative Biology, New Delhi, India

The role of two linked polymorphisms (G4C14 to A4T14) at TP73 and one polymorphism at MDM2 (309G>T) promoter were examined for the risk of leukoplakia and oral cancer. Further, a combined analysis was done with the previously studied codon72 pro/arg polymorphism at TP53. Moreover, the functional relevance of TP53 pro/arg polymorphism in the apoptotic property of the wild type and mutant TP53 gene was studied. Before embarking on the association studies, the distribution of 16 polymorphic variants at the TP53(7 SNPs), TP73(5 SNPs) and MDM2(4 SNPs) genes were estimated in 24 different subpopulations varied geographically and linguistically across India, as a part of the Indian Genome Variation Consortium Project. The allele, genotype and haplotype frequencies vary significantly among the different linguistic groups for some of the studied SNPs and the difference was also observed among the populations from different geographical locations. Next, TP73 G4C14 to A4T14 and MDM2 309G>T polymorphism were determined in 197 leukoplakia patients, 303 oral cancer patients and 319 healthy controls. The TP73 (GC/AT) genotypes increased the risk of leukoplakia (OR = 1.6, 95% CI = 1.1–2.3) and oral cancer (OR = 2.4, 95% CI = 1.7–3.3) but 309G>T MDM2 polymorphism independently could not modify the risk of any of the diseases. Stratification of the study population with different tobacco habits showed that the risk of the oral cancer is not modified further with the TP73 genotype. A combined analysis was done with the previously published data on TP53 codon72 pro/arg polymorphism. Pairwise genotype combination analysis revealed increase in risk for specific TP73-MDM2 and TP73-TP53 genotype combinations. Finally, the combined analyses taking polymorphisms at three loci revealed that the presence of at least one risk allele at all three loci increases the risk for both leukoplakia and oral cancer. Previously, the arg/arg genotype of TP53 codon72 was found to be the risk genotype for developing leukoplakia, but not in oral cancer. As the mutants of TP53 have impaired transcriptional

transactivation potential, thus instead of investigating the growth arrest mediated by TP53, efforts were directed towards analyzing the apoptotic potential of these Arg and Pro varieties of mutant TP53 protein. A sharp difference was observed between the conformational and the non-conformational mutants of TP53, which is not influenced by its different polymorphic variants.

422: Genetic susceptibility to gallbladder cancer: role of polymorphisms in candidate genes

B. Mittal, R. Mittal, S. N. Pandey, A. Srivastava, M. Vishnoi, K. Srivastava, M. Singh, G. C. Choudhuri

Sanjay Gandhi PGIMS, Lucknow, India

Introduction: Gallbladder cancer (GBC) is a highly fatal disease with poor prognosis. North India has one of the highest incidences of GBC, particularly in females, in the world. One of the common approaches to identify susceptible genes involves association studies in candidate genes. During past 5 years, we have evaluated association of cancer phenotype with candidate genes belonging to xenobiotic metabolism, lipid transport; bile acid synthesis, inflammatory and DNA repair pathways in a cohort of GBC patients.
Methods: The study involved 171 cases of GBC and 221 healthy controls from North India. The genotyping was done in peripheral blood DNA, using PCR-RFLP and DNA sequencing.
Results: In xenobiotic metabolism genes, significant risk of cancer was observed with slow allele of NAT2 ($p = 0.000007$; OR 3.4, 95% CI = 1.9–5.7), ile/val genotype of GSTP1 ($p = 0.013$, OR = 1.9, 95% CI = 1.1–3.1) and TC genotype of CYP1A1 Msp1 (OR 4.1, 95% CI = 1.3–11.9, $P = 0.012$) polymorphisms. CYP7A1 is involved in cholesterol and bile metabolism and a polymorphism -204 A>C conferred significant risk ($p = 0.005$; OR = 2.78; 95% CI: 1.3–5.6) for GBC and the risk was independent of gallstone pathway. The ABCG8 Asp/His polymorphism influencing intestinal cholesterol absorption and bile acid of gallbladder was found to be a high risk factor ($p = 0.011$; OR = 1.79; C = 1.1–2.8) for GBC. In addition, an important risk ($p = 0.025$; OR = 2.93; 95% CI 1.14–7.51) for GBC was also observed with Cys/Cys genotype of OGG1, the gene involved in base excision repair pathway of DNA. However, a haplotype consisting of two polymorphisms in XRCC1 (399G>A and194C>T) conferred low risk for GBC ($p = 0.002$; OR = 0.59; 95% CI = 0.42–0.82). Some important genetic variants like null alleles of GSTM1 and T1, AB/BB genotypes of GSTM3, C677T MTHFR did not influence genetic susceptibility for GBC. Significantly, genetic variants of IL1 and TNF alpha only influenced GBC risk in a sex-specific manner. Furthermore, a significant gene-gene and gene-environment interaction was observed between genetic variants and environment risk factors like tobacco. Presently, we are in the process of using whole genome SNP arrays to look for other candidate genes for GBC predisposition.
Conclusions: Studies suggest the involvement of various genetic variants which confer high risk of GBC. These studies are likely to provide clues to molecular markers which can significantly impact on our understanding of the pathobiology of GBC. Grant support from UPCST, ICMR, DST and CSIR.

423: MLH1 -93G>A promoter polymorphism and risk of head and neck squamous cell cancer

[1]**Pinaki Mondal**, [1]Debasish Boral, [2]Jayanta Chakrabarti, [3]Anup Roy, [4]Chinmay K. Panda, [4]Susanta Roychoudhury

[1]Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata 700032, India, [2]Cancer Centre Welfare Home and Research Institute, M.G. Road, Thakurpukur, Kolkata 700063, India, [3]Department of Pathology, Medical College and Hospital, 88 College Street, Kolkata 700072, India, [4]Department of Oncogene Regulation, Chittaranjan National Cancer Institute, 37 S.P. Mukherjee Road, Kolkata 700026, India

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world and accounts for 30 to 40% of all cancers in India. A substantial fraction of HNSCC tumors progresses through the mutator pathway, which is characterized by defects in DNA mismatch repair genes such as MLH1 [mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*)]. It has been previously reported that a single nucleotide polymorphism (SNP) located at promoter region of MLH1 gene (-93G>A, rs 1800734) is associated with lung, breast and colorectal cancer and also with the increased risk of colorectal cancer in long term smokers. We genotyped this polymorphism in 140 patients with HNSCC and 330 normal population samples from different regions all over India. We used DNA from tumor and normal tissue of same patient and genotyped the -93G>A polymorphism by direct sequencing. Interestingly, we found that, a substantial number of patients (71) had wild type allele (G) in their normal tissue that changed to the risk allele (A) in the tumor tissue. This observation is found to be significant ($P \leq 0.001$). The population samples from all over India, however, showed 48% heterozygous genotype (AG) and thus found to be highly polymorphic in Indian population. When we collected the information about the habit of tobacco smoking in 140 HNSCC patients, we found the G to A change from normal to tumor tissue DNA is also significantly associated with the habit of tobacco smoking ($P \leq 0.05$). Thus, this -93G>A promoter polymorphism of MLH1 gene is behaving like a somatic change in HNSCC patients from normal to tumor tissue with increased risk in patients with prolonged tobacco smoking habit. We are now working on in vitro and in vivo expression analysis by cloning MLH1 promoter separately with two alleles (G and A) in a luciferase vector and expression of MLH1 in tumor and normal tissues of patients showed G to A change, respectively. We also study the Microsatellite Instability (MIN) status of respective patients who showed G to A change by allelotyping in six microsatellite markers, as impaired function of MLH1 is reported to cause MIN.

424: Clinical implications of array-CGH: detection and characterization of submicroscopic chromosomal alterations in complex diseases and its significance in genetic diagnosis and counseling

Sabita K. Murthy, Preenu S. Jacob, Mahmoud Taleb Al Ali

Genetics Center, Al Wasl Hospital, Department of Health Services, DHA, Dubai, United Arab Emirates

Array based comparative genomic hybridization (array-CGH) has dramatically changed the overall perspective of genetic diagnosis. Cryptic chromosomal imbalances that were earlier left undetected due to limitations of microscopic resolution are now being detected quite efficiently. Reports from large studies reveal that of the 50–60% cases of complex diseases that were earlier classified as idiopathic (unknown etiology), 15–25% of them in fact do have cryptic genomic imbalances, either as a micro deletion or duplication. We undertook array-CGH studies on some selected referred pediatric patients with complex diseases—multiple congenital anomalies and/or mental retardation (MCA/MR), who had normal chromosomes by routine cytogenetic and by fluorescence in situ hybridization (FISH) studies. Submicroscopic chromosomal alterations were detected in 19% (4/21) cases, which were further validated and characterized either by FISH or by multiplex ligation-dependent probe amplification (MLPA) studies. These

alterations include: (1) A 253 kb microdeletion in a child presented with severe neurological disorder and typical features of Angelman syndrome (AS). The microdeletion segment spans BP1-BP2 region of the PW/AS critical region at 15q11.2 and include four genes—NIPA1, NIPA2, TUBGCP5, and CYFIP1. Is it causative or a variant? (2) A case of tissue specific mosaicism with a marker chromosome in skin fibroblast in a child with multiple congenital anomalies who was clinically suspected of having Pallistar-Killian syndrome. The marker was characterized by array-CGH and FISH as inv dup(3q25.33). (3) Two case of unbalanced chromosomal rearrangements (with submicroscopic deletion and duplication) due to de novo cryptic translocation, in children with multiple congenital anomalies. These findings were extremely helpful in obtaining a precise genetic diagnosis in the respective cases and in providing with a better and informed genetic counseling to the families. Array-CGH has huge implications in clinical practice. It is becoming convincingly and rapidly acceptable because of its immense power of unfolding a wealth of hidden information that is greatly helpful in obtaining a precise genetic diagnosis, to delineate a better genotype-phenotype correlation, to provide appropriate genetic counseling, and finally offer improved patient care and management. Diagnostic and prognostic significance of array-CGH and implications of copy number variants will be discussed.

425: Molecular pathogenesis of dystonia: role of GCH1 gene in Indian patients

[1]**Tufan Naiya**, [2]Shyamal K. Das, [3]Kunal Ray, [1]Jharna Ray

[1]S. N. Pradhan Centre for Neurosciences, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India, [2]Bangur Institute of Neurosciences and Psychiatry, 52/1-A, Sambhunath Pandit Street, Kolkata 700025, India, [3]Indian Institute of Chemical Biology (CSIR), 3, Raja S.C. Mullick Road, Kolkata 700032, India

Dystonia is a movement disorder characterized by sustained muscle contraction, repetitive twisting movement and abnormal posture of different body parts. Dystonic movement can either be slow or rapid, can change during action or different postures and may be fixed in advanced stages. Pain and tremor are most common associated symptoms with the disease. Dystonia is classified based on anatomical distribution of symptoms, age of onset, etiology etc. Limb dystonia is the most prevalent subtype among the Indian patients with higher prevalence among the males. 'Dystonia plus' syndromes such as dopa responsive dystonia (DRD) are associated with other neurological symptoms such as parkinsonian features. DRD has been reported to be associated with mutation in GCH1 (GTP cyclohydrolase 1) gene and inherited as an autosomal dominant trait. However, de novo mutations have also been reported. The encoded protein catalyzes the initial step of BH4 biosynthesis from GTP. BH4 is a cofactor required for the synthesis of monoamines and nitric oxide. A total of 110 patients with dystonia and early onset PD (age of onset <40 years) were screened for variants in GCH1 gene. For this purpose, all six exons of GCH1 were amplified by PCR, subjected to SSCP followed by DNA sequencing of samples that showed aberrant band pattern in SSCP relative to the controls. A total of 6 nucleotide variants including nonsynonymous change and deletion were detected in three families. Family members harboring the same mutation showed variable clinical presentations. A patient who was misdiagnosed as cerebral palsy and could not get appropriate treatment for the last 25 years was found to be a compound heterozygote for mutations in GCH1. Treatment modality and genetic counseling were advised to the genetically confirmed patients and their family members based on the genetic findings. However, further studies are needed to identify other genetic or epigenetic factors responsible for the large variations of clinical symptoms among the carriers of GCH1 heterozygous mutations. To our best knowledge, this is the first mutation report in GCH1 gene from India. The study is supported by funding from Council of Scientific and Industrial Research (CSIR), Government of India.

426: Expression profile of SAC genes in HNSCC samples and study of transcriptional regulation of a key SAC gene, CDC20 upon DNA damage

Somsubhra Nath, Taraswi Banerjee, Susanta Roychoudhury

Indian Institute of Chemical Biology, 4, Raja SC Mullick Road, Kolkata 700032, India

The spindle assembly checkpoint (SAC) prevents premature sister-chromatid separation and mitotic exit. The major players of SAC are anaphase-promoting complex (APC), CDC20, Mad and Bub family proteins. There have been reports of differential expression of the SAC proteins in several cancers. We have studied the expression level of these SAC genes in Head and neck squamous cell carcinoma (HNSCC) and also in various cancer cell lines. We observed a consistent overexpression of CDC20 in all cancer cell lines and primary tumor tissues. We reported that over-expression of CDC20 resulted in defective SAC as determined by mitotic index. FISH analysis showed that ectopic expression of CDC20 resulted in aneuploidy and multinuclei formation. This led us to study the regulation of CDC20 expression. There are few reports of the tumor suppressor protein p53 to be involved in regulation of SAC function. Here, we investigated the role of p53 in CDC20 regulation upon DNA damage. Initially, we observed a dose-dependent decrease in endogenous CDC20 mRNA and protein levels in HeLa cells upon transfection with a wt.p53 expression vector. We validated this observation by treating HepG2 cells with increasing concentrations of 5-fluorouracil (5-FU), a DNA damaging drug, to stabilize endogenous p53. To establish whether CDC20 downregulation by p53 occurs at the transcriptional level, we showed that expression of p53 in HCT p53−/− cells dose-dependently inhibited luciferase activity from a CDC20 promoter-luciferase vector. Previous reports showed an indirect role of p53 in CDC20 repression working through the NFY and E2F response elements in the CDC20 promoter. However, using a bioinformatics approach, we identified a putative p53 binding site within the 1 kb promoter of CDC20 that we used in the promoter-reporter assay. This led us to hypothesize that in addition to the indirect role, p53 might directly inhibit the CDC20 promoter. Towards this end, we showed that p53 still inhibits the CDC20 promoter containing mutated NFY and E2F binding sites both individually and in combination. ChIP assay showed the presence of p53 in the transcription complex on the CDC20 promoter both in p53 overexpressed HCT p53−/− cells and in 5FU-treated HepG2 cells. Also, EMSA done with the putative p53 sequence incubated with 5FU treated HepG2 cell nuclear extract showed significant binding and a supershift when incubated with anti-p53 antibody, reinforcing the fact that p53 binds to the sequence.

427: Polymorphisms of HIF1A gene in Mexican patients with preeclampsia

[1]**S. Nava-Salazar**, [1]E. N. Sánchez-Rodríguez, [1]C. A. Mendoza-Rodríguez, [2]P. Cruz-Cruz, [1]M. A. Cerbón

[1]Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México, Mexico, [2]Instituto Mexicano del Seguro Social, Hospital de Ginecología y Obstetricia No. 3 La Raza, Ciudad de México, Mexico

Preeclampsia is a pregnancy-specific syndrome characterized by new onset hypertension, proteinuria and edema. This disease is a considerable obstetric problem and significant source of maternal and neonatal morbidity and mortality. Although the pathophysiology of preeclampsia remains undefined, placental ischemia/hypoxia are widely regarded as a key factor. The hypoxia inducible transcription factor, HIF1A, is overexpressed in placentae from women with preeclampsia and contributes to the dysregulation of numerous genes related with trophoblast invasion. P582S and A588T are variants of HIF1A that have been associated to high transcriptional activity in some types of cancer and are used as risk factor markers of the disease. In the present study, we explore the association of the allelic polymorphism P582S and A588T of HIF1A gene and the association with prevalence of preeclampsia in Mexican population. The molecular analysis of P582S and A588T alleles was performed by PCR and direct sequencing. A case–control study of risk factors is currently undertaken. The preliminary results suggest that the allelic variants, P582S and A588T, present a similar frequency, in Mexican than other populations in the exploratory study. However, we are increasing the size of the sample to establish the prevalence of these polymorphisms and its possible association with preeclampsia in our population.

428: Analysis of DNA damage: death receptor apoptotic pathway gene SNPs in sporadic breast cancer: an interactive study

[1]**Ranjana Pal**, [1]Sailesh Gochhait, [1]Shilpi Chattopadhyay, [2]Pawan Gupta, [1]Rameshwar N. K. Bamezai

[1]National Centre of Applied Human Genetics, School of Life Sciences, Jawaharlal Nehru University, New Delhi 67, India, [2]Dharamshila Cancer Hospital and Research Centre, Dharamshila Marg, Vasundhara Enclave, Delhi 96, India

Apoptosis is central to the development and homeostasis of metazoans, and its dysregulation causes a number of human pathologies, including cancer, autoimmune diseases, and neurodegenerative disorders. Deregulated cell proliferation together with suppressed apoptosis constitutes the minimum common platform upon which all neoplastic evolution occurs. A cell has an elaborate machinery not only to repair the DNA damage but also to check the cell cycle till the repair is complete. If the damage is insurmountable, cell undergoes apoptosis. In many cancers, the pro-apoptotic proteins have inactivating mutations or the expression of anti-apoptotic proteins is upregulated, leading to unchecked growth of tumor and the inability to respond to cellular stress, harmful mutations and DNA damage. Altered expression or mutation of genes encoding key apoptotic protein could provide cancer cells with intrinsic advantage to survive and develop inherent resistance to chemotherapeutic drugs. We focused on the DDR-apoptotic pathway and sought for an association of SNPs in TP53 (Arg72Pro), BRCA2 (G-26A in 5′UTR), TNFSF10 (A-692G, C-716T, T-760C, C-802T), IL6 (C-253T, G-239C, G-237C, G-174T, C-126G), TGFB1 (C-1347T, exon 1 G-14C, C+29T, G+74C) and IFNG (Intron1 CA-repeat polymorphism) in a case-control study of 714 individuals (278 sporadic breast cancer patients and 436 controls) from north India, subsequently followed by SNP-SNP interaction analysis. The association study implicated TP53 Arg72Pro [P = 0.024, OR (95% CI) = 1.6 (1.1–2.5)], TNFSF10 T-716C [P = 0.041, OR (95% CI) = 0.6 (0.3–0.9)] and TGFB1 T-1347C [P = 0.020, OR (95% CI) = 0.6 (0.4–0.9)] polymorphisms with breast cancer. The interaction study indicated an increased protection under simultaneous presence of protector genotypes of BRCA2-TP53 [P = 0.014, OR (95% CI) = 0.4 (0.2–0.8)] (Gochhait et al., Breast Cancer Research, 9:R71), BRCA2-TGFB1 [P = 0.016, OR (95% CI) = 0.4 (0.2–0.8)], TNFSF10-TGFB1 [P = 0.044, OR (95% CI) = 0.5 (0.2–0.9)] and BRCA2-TNFSF10 [P = 0.009, OR (95% CI) = 0.5 (0.3–0.9)]. Similarly, the presence of risk genotypes of TP53-TGFB1 [P = 0.001, OR (95% CI) = 3.1 (1.6–6.2)] revealed an additive effect. Thus our study suggests that more elaborate interactive studies in future might help to gain further insight into the process of tumorigenesis as well as developing biomarkers for better prognosis and therapeutic interventions.

429: Molecular genetic, histopathological and clinical examinations for genotype–phenotype analysis in patients with TGFBI-linked corneal dystrophies

[1]**Preeti Paliwal**, [1]Arundhati Sharma, [2]Radhika Tandon, [2]Namrata Sharma, [2]Seema Sen, [2]Jaya Gupta, [2]J. S. Titiyal, [2,3]R. B. Vajpayee

[1]Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India, [2]Dr. Rajendra Prasad Centre of Opthalmic Sciences, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India, [3]Centre for Eye Research, Department of Ophthalmology, University of Melbourne, Australia

Purpose: Mutations in the transforming growth factor beta I (TGFBI) gene cause several types of autosomal-dominant corneal dystrophies. The role of this gene in north Indian patients and their family members affected by lattice and granular corneal dystrophies was investigated.
Methods: Forty two subjects from 31 unrelated families diagnosed with autosomal dominant lattice or granular corneal dystrophies were recruited. Corneal phenotypes were assessed by slit lamp examination followed by complete ophthalmic investigations. Genomic DNA was obtained from blood samples, and exons of the TGFBI gene were screened for mutations by direct DNA sequencing. Clinical and histopathological findings were compared with the molecular genetic findings for genotype–phenotype correlations.
Results: Typical missense mutations in exon 4 (R124C) and exon 12 (R555W) of the TGFBI gene were found in majority of the patients with clinical lattice corneal dystrophy (LCD) and granular corneal dystrophy (GCD) respectively. A novel mutation S516R was seen in two affected members of a family with GCD while T538P mutation, seen for the first time in India was found in a patient diagnosed with LCD. Five cases of Avellino corneal dystrophy with mutation R124H were also detected for the first time in India. Other mutations detected included R124L, H626R, and A546D. Two single-nucleotide polymorphisms, H428H and F540F were also found.
Conclusions: Mutational analysis of TGFBI gene in North Indian families affected by corneal dystrophy showed R124C and R555W TGFBI mutations to be the most common ones to cause lattice and granular corneal dystrophies in the families studied. Apart from these, one novel mutation in GCD and three variants of LCD have also been detected. Avellino corneal dystrophy confirmed by molecular analysis is being reported for the first time from India. Comparison of clinical, histopathological and molecular data showed a strong genotype–phenotype correlation in all the patients under study.

430: Chymotrypsin C gene polymorphisms predict susceptibility to tropical calcific pancreatits

[1]**Sumit Paliwal**, [1]Swami Prakash, [2]D. Nageshwar Reddy, [1]Giriraj Ratan Chandak

[1]Centre for Cellular and Molecular Biology, Hyderabad, India, [2]Asian Institute of Gastroenterology, Hyderabad, India

Background and aims: Tropical calcific pancreatitis (TCP) is a juvenile, severe, non-alcoholic form of chronic pancreatitis (CP) characterised by high incidence of diabetes mellitus, stone formation and pancreatic cancer. We have earlier established its genetic basis and showed it to be strongly associated with mutations in SPINK1 and cathepsin B genes rather than cationic trypsinogen mutations. Recently, chymotrypsin C (CTRC) variants that diminish its activity or secretion were found to be predicting susceptibility to chronic pancreatitis including TCP. Our study aimed to look for major CTRC variants in a large sample population using ethnically matched case–control approach.
Subjects and methods: We sequenced the exons 2, 3 and 7 of CTRC gene in 300 TCP patients including 140 fibro-calculous pancreatic diabetes and 150 controls and calculated the statistical significance of association of CTRC variants using χ^2 test.
Result: Out of the various reported polymorphisms in CTRC, we found 217G>A, 649G>C, 703G>A, 760C>T to be present in this study. CTRC variants were over-represented in TCP patients (35/300; 11.7%) compared to the controls (9/150; 6%) and 649G>C (p.G217R) was the most common variant (12/35, 33%). Of the 35 affected individuals, nine were homozygous for 703G>A while one patient carried both 217G>A and 703G>A variants. However, only 649G>C [OR= 3.6, 95% CI 1.06–13.39, p = 0.02] and 703G>A [OR = 3.84, 95% CI 1.16–14.01, p = 0.012] reached statistical significance. The commonly reported 738_761del24 variant in Caucasians was not observed in our population, while a novel frame shift mutation in exon 7 leading to a prematurely truncated protein was identified in one of the TCP patients.
Discussion: Our study on a large cohort of TCP patients confirms that CTRC variants may play a significant role in the pathogenesis of TCP. The novel variant, 649G>C (p.G217R) is also likely to affect CTRC activity, since 649G>A (p.G217S) has been shown to have significantly impaired catalytic activity. CTRC is known to regulate auto activation of cationic trypsinogen and hence these variants may act as the trigger that is otherwise missing in TCP patients since cationic trypsinogen mutations have not been identified in TCP. Thus, our findings demonstrate that CTRC is a new candidate gene for tropical calcific pancreatitis.

431: Candidate tumor suppressor genes in the short arms of chromosomes 3 and 9 differentially associate with the development of head and neck squamous cell carcinoma

[1]**Chinmay K. Panda**, [1]Susmita Poddar, [1]Amlan Ghosh, [1]Guru Prasad Maiti, [2]Anup Roy, [1]Anirudha Dam, [3]Bidyut Roy, [4]Susanta Roychoudhury

[1]Chittaranjan National Cancer Institute, 37 S.P. Mukherjee Road, Kolkata 26, India, [2]Calcutta Medical College and Hospital, 88 College Street, Kolkata 73, India, [3]Indian Statistical Institute, 203 Barrackpore Trunk Road, Kolkata 35, India, [4]Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata 32, India

Head and neck squamous cell carcinoma (HNSCC) is one of the prevalent cancers in India and its subcontinent. Statistical analysis based on age specific incidence suggested that this type of tumor arise following five to ten independent genetic alterations. Deletion analysis identified association of chromosomal 3p12.3, 3p21.31, 3p22.1, 9p21-22, 9q22.3 and 11q22.3-24 regions with dysplastic head and neck lesions. On the other hand, deletions in the chromosomal 3p14.3, 3p22.3, 8p21.3-23, 9p23-24, 9q11-13 and 11p13-15 regions were seem to be associated with HNSCC. Among these deleted regions, high-resolution deletion mapping was done in 3p21.31, 3p12.3 and 9p21-22 regions to find out the candidate tumor suppressor genes. High frequencies of deletions have been seen in three discrete areas in chr. 3p21.31 (D3A, D3B and D3C), one in 3p12.3 (D3D) and two in 9p21-22 (D9A and D9B). Among these highly deleted regions, D3B, D3D and D9B regions showed comparatively high frequency of deletions (48–57%) in both dysplastic head and neck lesions and invasive head and neck squamous cell carcinoma (HNSCC). The number of the candidate TSGs that are located in the highly deleted regions are as follows D3A (1.14Mb): LIMD1, LTF; D3B (0.82Mb): CDC25A, SHISA5; D3C (1.38Mb): RASSF1, CACNA2D2; D3D (0.83Mb): ROBO1; D9A (0.20Mb): SH3GL2; D9B (0.30Mb): CDKN2A (p14, p16) and CDKN2B (p15). The promoter methylation studies showed high frequency of methylation (52–69%) in the LIMD1 and LTF genes followed by CACNA2D2 (44%), SH3GL2 (41%), CDKN2B (27%), RASSF1 (22%), ROBO1 (20%) and CDKN2A (15%) in the dysplastic lesions. Similar trends have also been seen in the HNSCC samples. No mutation has been seen in the CDKN2B gene. High frequency of mutation (18%) has been seen in the CDKN2A gene followed by SH3GL2 (10%), CDC25A (10%), LIMD1 (7%) and LTF (2%). Our data suggests that multiple candidate TSGs like LIMD1, LTF, ROBO1, Endophillin and CDKN2A gene are associated with mild dysplastic lesions of head and neck, whereas RASSF1, CACNA2D2, CDC25A and CDKN2B are associated with progression of dysplastic lesions. The expression (RNA/protein) analysis of these genes showed significant reduced expression in HNSCC samples indicating these as candidate TSGs. Thus, it seems that multiple pathways like endophillin associated EGF receptor degradation, deregulation of G1/S cell cycle checkpoint and inhibition of apoptosis are associated with the early dysplastic lesion of head and neck.

432: Role of arsenic in blood cancer

Sonali Paul, Tulika Chakraborty, Ajanta Halder, Debasish Banerjee, Jaysree Banerjee, Madhusnata De

Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, 99 Sarat Bose Road, Kolkata 700026, India

Cancer is a class of disease or disorder characterized by uncontrolled division of cells and the ability of these cells to spread, either by direct growth into adjacent tissue through invasion, or by implantation into distant sites by metastasis—where cancer cells are transported through the bloodstream or lymphatic system. The major forms of blood cancer are leukemia, lymphoma and multiple myeloma. These cancers are formed either in the bone marrow or the lymphatic tissues of the body. They affect the way the body makes blood and provides immunity from other diseases. Arsenic is a naturally occurring metalloid that has been associated with increased incidence of human cancer in certain highly exposed populations. Arsenic is released to the environment by natural means such as solubilization from geologic formations into water supplies. It is also released to occupational and community environments by such activities as nonferrous ore smelting and combustion of fuels containing arsenic. Several lines of evidence indicate that arsenic acts indirectly with other agents to ultimately enhance specific genotoxic effects that may lead to carcinogenesis. Millions of persons around the world are exposed to low doses of arsenic through drinking water. Chronic arsenic poisoning remains a public health crisis in West Bengal. Over here, many persons have been exposed to arsenic from drinking water over a significant period of time. Arsenic has already been documented as a lung, bladder and skin carcinogen in humans. The present work aimed to study the role of arsenic in blood cancer. We studied the chromosomal aberrations in the leucocytes of both blood cancer patients as well as in age and sex matched healthy controls. We found

a greater incidence of chromosomal aberrations in blood cancer patients as compared with the healthy controls. This correlated to the increased arsenic concentration in the hair and nail samples of the patients as compared with healthy individuals. Reactive oxygen species and other free radicals are known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. We also studied the blood levels of glutathione peroxidase and superoxide dismutase in both patients and healthy individuals and found significant decrease in both enzyme levels in patients indicating significant changes in antioxidant defense system in blood cancer patients. Thus our work indicates a role of arsenic as a predisposing factor for blood cancer.

433: SLE in Asia: genetic insights towards better management

[1]**Maude Elvira Phipps**, [2]Yusmin Yusuf, [3]Swan Sim Yeap

[1]Monash University, School of Medicine and Health Sciences, Sunway Campus, Malaysia, [2]University of Malaya, Faculty of Medicine, Kuala Lumpur, Malaysia, [3]Subang Jaya Medical Centre, Subang, Selangor, Malaysia

Systemic lupus erythematosus (SLE) is an enigmatic autoimmune disease which predominantly affects women worldwide. Being multifactorial in nature, the disease is manifested through the interplay of numerous genetics and environmental factors. Although this disease is currently diagnosed using the ARA set of criteria, the dynamics of impaired immune regulation and differential biomarker presentations at different stages of the disease still serve to confound prognosis. In the absence of firm diagnostic and prognostic indicators, the treatment of SLE is managed with the use of steroidal and more recently non-steroidal therapies. Better ways of management are being investigated. To date, several genes have been shown to be major players in precipitation and molecular pathology of SLE. Female to male ratios have been reported to be as high as 10:1 and SLE manifests in Caucasians, Africans and Asians. In Asia, SLE seems most prevalent in the Chinese compared to other ethnicities, as evidenced by numerous reports including our previous publications. We report that in the Chinese, Malay and Indian patients that we have investigated, genetic variants of HLA Class I and II and cytokines IL1R1, IL6, IL2, IL4, IL10 play significant roles in the molecular pathology of the disorder and its complications such as lupus nephritis. We present evidence to show predisposition to and protective effects of some of these variants and also ethnic specificities. The latest findings will also be discussed.

434: Spectrum of chromosomal imbalances associated with mental retardation

Rajitha Ponnala, Usha Dutta, Ramadevi A. Radha, Ashwin Dalal

Diagnostic Division, Centre for DNA Fingerprinting and Diagnostics, CDFD Interim Building, ECIL Road, Nacharam, Hyderabad, India

Chromosomal imbalance involves full aneuploidy (gain or loss of a whole chromosome) or partial aneuploidy (part of a chromosome) in mosaic or non-mosaic state. Loss of chromosomal material generally has more devastating effect on the growth of the conceptus than does an excess of material (i.e., trisomy). Survival imbalance produces a phenotype of wide spread dysmorphogenesis and there may be malformation of internal organs and limbs. The most complex organ of all, the brain is the most vulnerable to a less than optimal genetic constitution and some compromise of mental and intellectual functioning, usually to the extent of an obvious deficit. There are reports of sex chromosomal abnormalities including XXY, XYY, and fragile X karyotypes in individuals, but structural autosomal defects have rarely been reported. This paper presents patients with autism, mental retardation, minor dysmorphic features, and structural autosomal defects. These patients shared autistic features including fascination with inanimate objects, catastrophic reactions to changes in their environment or their daily routine, echolalia, and poor relatedness; IQ scores indicate mild to severe retardation. Their autosomal abnormalities included deletions of 8p, 13q and 18q, and so on. Chromosomal analysis should be performed on mentally retarded, autistic individuals, especially those with minor physical anomalies and no specific etiology for their retardation.

435: Nectin-like molecule 1 is a glycoprotein with a single N-glycosylation site at N290KS which influences its adhesion activity

Boqin Qiang, Li Zhao, Zhiqiang Zhao, Tao Chen

National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, National Human Genome Center, 5 Dong Dan San Tiao, Beijing 100005, China, China

Nectin-like molecule 1 (NECL1) is a neural tissue-specific immunoglobulin-like cell–cell adhesion molecule which has Ca^{2+}-independent homo- or heterophilic cell–cell adhesion activity and plays an important role in the formation of synapses, axon bundles and myelinated axons. Here we first detected the expression of NECL1, encoded by the CADM3 gene in human fetal and adult brains, and mouse brains at different developmental stages. The results indicated that two bands with molecular weights of about 62 kDa and 48 kDa were found in human fetal brain, while only one band with a molecular weight of about 48 kDa was found in human adult brain; two bands with molecular weights of about 62 and 48 kDa whose expression level gradually increased were also found from mouse E16 to P14, while only one band with a molecular weight of about 48 kDa was found from P14. Bioinformatics analysis showed there were two putative N-glycosylation sites within human NECL1 at positions N25LS and N290KS and within mouse Necl1 at positions N23LS and N288KS, respectively. There was no O-glycosylation site in either human NECL1 or mouse Necl1. Based on the results of N-glycosidase F treatment with human fetal brain tissue and lysates from transient transfection with human wild-type or glycosylation site mutant NECL1 in 293ET cells, we demonstrated that human NECL1 is an N-linked glycoprotein with a single glycosylation site at position N290KS. Cell aggregation assay further showed there was an increased adhesion activity after the glycosylation site mutation of NECL1 molecule.

436: Maternal grandmothers with advance-age reproduction are more likely to have Down syndrome grandchildren

[1]**Nallur Basappa Ramachandra**, [1]Suttur S. Malini, [2]Mysore R. Savitha

[1]University of Mysore, Human Genetic Laboratory, Department of Studies in Zoology, Manasagangotri, Mysore 570 006, India, [2]Mysore Medical College, Department of Pediatrics, Cheluvamba Hospital, Mysore, India

Chromosomal syndromes are significantly contributing to reproductive failure, birth defects, mental retardation, delayed puberty, hermaphrodites in humans. Down syndrome (DS), trisomy 21 is the most common chromosomal syndrome that affects one in 600–800 live births. The advanced maternal age is the only well known risk factor to cause DS. Our study of 150 DS cases revealed that many young mothers produced DS children than advanced age mothers in India. Logistic regression between the case-control studies indicated that the maternal grandmothers (78%) had advanced age during conception of their daughters who gave birth to a DS child. Therefore, it is important to sort out the effect of advanced age mothers vs grandmothers on increased frequency of DS reported in different populations. The implications of this finding for disease management and population health will be discussed.

437: Prevalence of iron deficiency and haemoglobinopathies in the population

Tapash Rudra, Sila Chakrabarti, Bani Sengupta, Geeta Talukder

Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road Kolkata 700026, India

Nutritional Anaemia is very common in India, while iron deficiency is a well recognized cause of NA. Iron deficiency is the most common dietary deficiency in the world. In our present study we took 113 patients attending haematology outdoor and subsequently referred to our unit.

The patient samples were divided into three groups—(1) CONTROL GROUP with Hb 11 g/dl or more and transferrin saturation >16%; (2) IRON DEFICIENCY GROUP with Hb 11 g/dl or more and transferrin saturation <16%; and (3) IRON DEFICIENCY ANAEMIA GROUP with Hb less than 11 g/dl and transferrin saturation <16%. Furthermore, we also studied the prevalence of genetic anaemias and other haemoglobinopathies in these three groups.

Results showed (1) Hb, MCV, MCH and transferrin saturation significantly lower in ID & IDA Groups compared to Control Group. (2) Hb, MCV, MCH and transferrin saturation significantly lower in IDA Group compared to ID Group. Again, the prevalence of genetic anaemias including alpha and beta thalassaemia and presence of other haemoglobinopathies are significantly higher in ID and IDA Groups compared to Control Group. So we can conclude that nutritional anaemia especially iron deficiency is fairly common in the general population and prevalence of genetic anaemias is significantly higher together with iron deficiency.

438: Molecular pathogenesis of Parkinson's disease: role of DJ1 and LRRK2 genes in Indian patients

[1]**Tamal Sadhukhan**, [2]Shyamal K. Das, [1]Jharna Ray

[1]S. N. Pradhan Centre for Neurosciences, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India, [2]Bangur Institute of Neurosciences and Psychiatry, 52/1-A, Sambhunath Pandit Street, Kolkata 700025, India

Parkinson's disease (PD) is one of the most common neurodegenerative disorders which affects more than 1% population over age 60. PD is a complex disease, both environmental and genetic factors play significant role for its pathogenesis. Cases are mostly sporadic, about 10% has been found to be familial. A total of 12 chromosomal loci and 8 underlying genes have been identified for autosomal dominant and recessive traits of PD. We investigated potential roles of DJ-1 and LRRK2 (Leucine rich repeat kinase 2) genes in Indian PD patients. While DJ-1 has been linked to autosomal recessive early onset form of PD, LRRK2 has been implicated in autosomal dominant form of the disease. We screened a total of 306 patients with mean age of onset 48 ± 13 (age range, 10–77 years) from 291 unrelated families. The DJ-1 gene was screened by polymerase chain reaction, single stranded conformation polymorphism and DNA sequencing. A total of nine nucleotide variants were detected including one reported nonsynonymous change in exon 5 (R98Q) in three isolated patients. In addition, six SNPs in non-coding region, one 18 bp deletion in intron 1 and a novel single nucleotide insertion in Intron 4 were also detected. The 18 bp deletion is highly polymorphic, and was found both in patients and controls in similar proportion (13.75 vs. 11.2%). Currently we are examining controls for other variants identified in DJ-1 of the patients for potential association of the variants with the disease. In case of LRRK2, a large gene with 51 exons, initially we aimed to screen for sequence variants in the regions of the gene where mutations have been reported. So far we have identified only three nucleotide variants that are reported in dbSNP. Further studies are in progress. This study is supported by UGC and CSIR, Government of India.

439: GST M1 and GST T1 gene deletion polymorphisms in chronic myeloid leukemia

[1]**K. Sailaja**, [1]D. Surekha, [1]D. Nageswararao, [2]D. Raghunadharao, [1]S. Vishnupriya

[1]Department of Genetics, Department of Genetics, Osmania University, Hyderabad, India, [2]Nizams Institute of Medical Sciences, Panjagutta, Hyderabad, India

The glutathione S-transferase (GST) family of enzymes play a crucial role in biotransformation of environmental carcinogens, pollutants, drugs and other xenobiotics. Polymorphisms in GST genes have been shown to be associated with susceptibility to various diseases and their outcome.

The present study was done to find out whether these deletion polymorphisms of GSTT1 and GSTM1 were associated with risk of developing chronic myeloid leukemia (CML).

Our study includes 150 chronic myeloid leukemia cases and 150 age and sex matched healthy controls. Deletion polymorphisms of GSTM1 and GSTT1 were analyzed by multiplex polymerase chain reaction followed by agarose gel electrophoresis.

Our results showed increased frequency of homozygous GSTT1 null genotype (GSTT1*0/GSTT1*0) (18%) in the CML patients when compared to control group (13.33%) (OR: 4.8148, CI: 3.1809–7.2879), whereas no significant association was found with GST M1 null genotype. With respect to sex of the proband, females had higher frequency of GSTT1 (21.27%) and GSTM1 (34.04%) genes. GSTT1 null genotype frequency was increased in patients with advanced phase (27.27%) of the disease as compared to chronic and accelerated phase (17.18%, 18.18%). When genotypic frequencies were compared with respect to clinical responses (hematological and cytogenetic), GSTT1 null genotype (26.31%) was found to be elevated only in hematological poor responders compared to complete/partial responders (17.35%, 10%). When both the null genotypes were considered, the GSTM0T0 frequency was found to be elevated in hematological poor responders (10.52%) as well as in cytogenetic poor responders (8.10%). This study suggests that GSTT1 null genotype might be associated in conferring risk for CML development and disease progression. Deletion polymorphisms of GSTT1, M1 with altered gene functions also play a role in response to treatment with chemotherapeutic drugs.

440: Different domains of SIN3B interact with various co-repressors

Daman Saluja, Harish Batra, Vineet Kumar, Mashook Ali, Nidhi Bansal, Leena Vig

Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India

Various transcriptional repressors such as Mad, RxR, MeCp2, estrogen receptor, RPX and Pit1 recruit mSIn3A-HDACs complex leading to repression of transcription. The first Sin3 to be cloned and sequenced was from the budding yeast (ySin3). Mouse has two isoforms, mouse Sin3A and mouse Sin3B, both showed significant homology to ySin3, highest being in four PAH domains and region between PAH3 and PAH4 (called HID domain) and in highly conserved domain (HCR). There is only one Sin3 homologue annotated in Drosophila although it may exist in as many as four splice variants. There is also evidence for splice variants of human SIN3A. In the present study, we characterized human SIN3B and its function. The 3390 bp long orf of SIN3B sequence encodes for a 1130 amino acid long polypeptide (AY706204). The gene for SIN3B contains 19 exons and spreads over 50 kb of genomic DNA on chromosome 19. The protein shares 40% overall homology to ySin3 protein and is more homologous to mouse Sin3B (about 90%) than to mouse Sin3A. It also shows four paired amphipathic helix (PAH) domains, HID and a highly conserved domain. Using co-immunoprecipitation technique, we have shown that SIN3B is a part of the core complex containing SIN3A and HDACs. Human SIN3B is specifically phosphorylated at tyrosine. While phosphorylated SIN3B co-immunoprecipitated with SIN3A, HDAC3, and Mad1 protein, the SIN3B associated with N-CoR, HDAC4 and HDAC1 was hypo-phosphorylated suggesting phosphorylation is important in determining its interaction with proteins. The leukemic associated eight- twenty-one RUNX1T1 (ETO/MTG8) and its homologue CBFA2T2 (MTGR1) (Myeloid transforming gene-related protein 1) are putative transcriptional repressor proteins. The interaction of MTG8 and CBFA2T2 were analyzed with SIN3B protein using yeast two hybrid and co-immuno-precipitation. Amino terminal domains of SIN3B were found to interact with MTG8 and CBFA2T2 suggesting that ETO homologues may repress transcription by recruiting SIN3B-HDAC complex during early hematopoietic differentiation. We further observed that tumor repressor proteins such as Mad and p53 also interact with SIN3B. Using yeast two hybrid technique, we established that amino terminal domain of SIN3B was essential and sufficient for its interaction with Mad1 and p53 protein. Deletion mutants of amino terminal region of SIN3B suggested that although PAH2 domain is essential, but is not sufficient for interaction with Mad1. In contrast, region between PAH2 and PAH4 of SIN3B interacts with p53 protein.

441: Association of vitamin D receptor polymorphisms with myopia development

[1]**A. Sandhya**, [1]Ch Hema Bindu, [2]K. Prasad Reddy, [1]S. Vishnupriya

[1]Department of Genetics, Osmania University, Hyderabad, India, [2]Maxivision Eye Hospital, Begumpet, Hyderabad, India

Myopia (near-sightedness) is a common, sight threatening, multifactorial ocular disorder exhibiting genetic heterogeneity, in which retinal defocus results in impaired vision. During myopia progression, vitamin D receptor (VDR) gene, mapped to chromosome 12q12-14(near myopia locus), plays a key role in phosphate metabolism, cell differentiation and proliferation, alters intracellular calcium levels resulting in defective neuromuscular transduction in the eye. It serves as a positional and functional candidate gene for myopia. It constitutes Fok1, Bsm1, Taq1 polymorphisms which seem to affect the transcriptional efficiency and functionality of the protein. Hence, we made an attempt to analyze these gene polymorphisms through PCR-RFLP method to understand its association and functional significance in disease susceptibility. In our present case-control study (210 myopia cases and 190 normal age and sex matched healthy controls), gene and genotype frequencies of the 3 gene polymorphisms were calculated and statistical comparison was made with respect to sex of the proband, age at onset, type of myopia, parental consanguinity and familial incidence. The genotype distribution of Fok1 polymorphism ($\chi^2 = 4.02$) showed significant increase of ff genotype frequency in male probands (13.43%), low myopia cases (26%), non-familial cases (15%), consanguineous cases (11.32%) and in later age (>20 years) at onset cases (32%). Taq1 polymorphism showed no significant genotypic association ($\chi^2 = 1.02$). In contrast, genotype distribution of Bsm1 polymorphism showed a significant increase of Bb frequency in disease cases (45%) compared to controls (36.31%) with insignificant allele distribution compared to controls. However, elevation of B allele frequency was found in females (0.632), non-consanguineous cases (0.623), familial cases (0.618), early age at onset cases (0.641) and high myopia cases (0.65). Possible correlations of VDR polymorphic genotypes revealed that individuals (62%) with B, T and f haplotypes had progressive myopia. In conclusion, our results indicate that 'B' allele of Bsm1 polymorphism confers risk to myopia causation whereas 'T' allele of Taq1 and ff genotype of Fok1 polymorphism are associated with other genetic/non-genetic factors during myopia progression.

442: Phenome scan (genotype/dense phenotype association studies) of a comprehensive clinicopathological database derived from large numbers of autopsy cases

[1]**Motoji Sawabe**, [1]Tomio Arai, [2]Shinobu Ikeda, [2]Masaaki Muramatsu, [3]Makiko Mieno, [4]Noriko Tanaka

[1]Tokyo Metropolitan Geriatric Hospital, 35-2 Sakae-cho, Itabashi, Tokyo, Japan, [2]Tokyo Medical and Dental University, 2-3-10 Kanda-surugadai, Chiyoda,Tokyo, Japan, [3]Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi, Japan, [4]Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, United States of America

Background: Since most genes are expressed in more than one tissue and engaged in many functions, some genetic variant could be associated with unpredicted diseases with or without interactions with other genetic variants or environmental factors. Recently Pembrey and Jones proposed a new method, 'phenome scan' (analogous to 'genome-wide scan', but in an opposite direction), in which dense phenotypic information in human cohorts is scanned for associations with individual genetic variants. We attempted to apply this method to our comprehensive clinicopathological database derived from large numbers of autopsy cases.
Subjects: The subjects of this database derived from more than 2,000 consecutive autopsies from the patients died in a community-based geriatric general hospital during recent 12 years. Most of the cases were registered in a SNP database named 'A Database of Japanese Single Nucleotide Polymorphism for Geriatric Research (JG-SNP)' and freely accessible on the Internet at http://www.tmgh.metro.tokyo.jp/jg-snp/english/E_top.html.
Results: The average age of the patients was 80 years and male to female ratio was 1.2. The autopsy rate has declined from 46% to 21%

during this period. The database contained patient information, histories of smoking and drinking, clinical diagnosis including 26 geriatric diseases, serum lipid data, 750 pathological findings, 42 major pathological diagnoses, atherosclerotic degrees of 10 major arteries, emphysematous degrees, and so on. Twenty-six geriatric diseases included 6 cardiovascular diseases, 3 neurological diseases, 5 metabolic and skeletal disease, 3 respiratory diseases, 4 malignancies and so on. Forty-two major pathological diagnoses included an extended and partially overlapping list of geriatric diseases. Both clinical and subclinical findings were registered among 750 pathological findings. DNA was extracted from unfixed renal tissue in all cases and used for molecular analysis. The results of application of this method to several genes such as CAR, NOS3 and PRKCH will be presented.
Conclusions: In contrast to physiological limitation of human genome (3 gigabase), human phenome is unlimited and difficult to define. Although the number of the subjects registered in this database and available medical information were limited, this database was still useful for the phenome scan to identify significant associations between genetic variant, especially for the genes of unknown functions, and geriatric diseases or pathological conditions.

443: PCR-based detection of Southeast Asian (–Sea) and Filipino (–Fil) alpha-thalassemia deletions in Filipino patients

[1,2]**Catherine Lynn Silao**, [3]Ernesto Yuson, [3]Lisa Naranjo, [3]Len Leporgo, [1,2]Carmencita Padilla

[1]Institute of Human Genetics, National Institutes of Health Philippines, Manila, Philippines, [2]Department of Pediatrics, University of the Philippines—Philippine General Hospital, Manila, Philippines, [3]Fe del Mundo Children's Medical Center, Quezon City, Philippines

Background: Alpha-thalassemia is the most common single-gene disorder in humans, stemming from mutations in the alpha-globin gene. The most common of these mutations are deletions which remove one or both of the two functional alpha-globin genes. Severity of disease characteristics depend on the number of deleted genes. Carriers of single- and double-gene deletions may either be asymptomatic or exhibiting mild microcytosis, with or without anemia, while those with three alpha-globin genes deleted suffer from Hb H disease which is characterized by a moderately severe hemolytic anemia with a variable clinical course. Deletion of all four alpha-globin genes lead to severe intrauterine anemia, resulting in fetal death.
Methodology: To determine the molecular basis of alpha-thalassemia in Filipino patients, genomic DNA samples from 49 clinically diagnosed alpha-thalassemia patients were analyzed for the most common two-gene (cis) deletions found in Southeast Asian populations. Screening of these common deletions was done by polymerase chain reaction using primers flanking the deletion breakpoints of the Southeast Asian (–SEA) and Filipino (–FIL) deletions.
Results: Of the 49 samples analyzed, 10 were found positive for the Southeast Asian (–SEA) deletion while 9 were found to have the Filipino (–FIL) deletion.
Conclusion: Our results reveal a prevalence pattern similar to other Southeast Asian populations, with the SEA deletion being the most prevalent and the Filipino deletion also showing a high incidence. Determination of the alpha-thalassemia mutations in Filipinos is useful in the genetic counseling and prenatal diagnosis of this disease.

444: Analysis of methylation of 5′-flanking region of Fmr-1 gene and its modulation by sex steroids hormones in aging mouse brain

[1,2]**Kanchan Singh**, [1]S. Prasad

[1]Banaras Hindu University, Department of Zoology, Varanasi 221005, UP, India, [2]Dr. BR Ambedkar Center for Biomedical Research, North Campus, University of Delhi, Delhi 110007, India

Epigenetic mechanisms of the transcriptional regulation require covalent modifications of DNA and therefore chromatin organization that alter patterns of gene expression. DNA methylation is one of such mechanisms, which has been associated with neuronal cell differentiation and also implicated in learning and memory. Methylation of cytosine in CpG rich sequences plays an important role in the organization of eukaryotic genome and it may be helpful for understanding the mechanisms of genome function. The 5′ upstream region of the human FMR1 gene that includes the trinucleotide repeat and its immediate flanking regions constitute a CpG island. Heavy methylation of this island has been described which in turn silence the FMR1 transcription in the brain of fragile X mental retardation syndrome. Several reports indicate sex steroid hormones modulate the levels of methylation at promoter region. In the present work we have studied the methylation status of mouse Fmr-1 5′ flanking sequences by using methylation sensitive restriction enzymes MspI and HpaII. MspI and HpaII recognize 5′-CCGG-3′ sequences. HpaII cannot cleave if the internal C is methylated i.e. 5′-CmCGG-3′. MspI cleaves the sequence irrespective of methylation. The experimental mice were taken from Young (6 ± 2W), adult (20 ± 5W) and old (60 ± 5W) ages. Only adult and old mice from both the sexes were used for sex steroid hormones related study. These hormones (Testosterone and 17b-Estradiol) were administrated separately to gonadectomized mice by intra-peritoneal injection. Results obtained from our study clearly reveal that Fmr-1 5′ flanking region undergoes hypermethylation as a function of age in both the sexes. The sex steroid hormones testosterone and 17b-estradiol have ability to modulate the methylation of Fmr-1 gene in the mouse brain differentially as a function of age in both male and female. The present finding is novel and it has implications on fragile X mental retardation syndromes, especially the FXTAS and their potential therapeutic intervention with steroid hormones.

445: Androgen signaling and androgen-related gene polymorphisms and the risk prostate carcinoma

[1]**Abha Soni**, [1]Anju Bansal, [2]N. K. Mohanty, [1]Sunita Saxena

[1]Institute of Pathology, ICMR, Safdarjung Hospital Campus, New Delhi, India, [2]Safdarjung Hospital, New Delhi, India

Background: Knowing the widespread prevalence of prostate cancer (PCa) in the world and palliative nature of treatment after the disease spreads, the search for reliable molecular biomarkers is as important as ever. Molecular markers that can accurately predict the presence, metastasis and serve, as prognosticators are desired to foretell the course of PCa. In the context of hormone-sensitive disease PCa, genes involved in hormone interactions are likely candidates for disease susceptibility. By regulating cell proliferation, differentiation and apoptosis the androgen receptor (AR) plays a pivotal role in PCa progression, as well as in normal prostate development. AR acts as a transcription factor regulating the prostate-specific antigen (KLK3) gene by interactions with AREs. The CYP19A1 gene encodes aromatase, enzyme that converts androstenedione/testosterone to estrone/

estradiol, low levels of E2 have been proposed to be associated with PCa risk. Given the plethora of candidate PCa molecular targets, the present study aims to examine the impact of polymorphisms in androgen signaling and androgen-related genes, AR, KLK3 and CYP19A1 on PCa risk.
Materials and methods: Blood samples and FFPE tissue were obtained for DNA analysis. The [CAG] and [TTTA] repeats in AR and CYP19A1 genes, respectively, were genotyped by GeneScan analysis. SNP in the 5′-UTRs of KLK3 (A>G at -158) gene was detected by PCR-RFLP assay.
Results: Our results on AR CAG in PCa and controls showed slight significance ($p = 0.059$), when analysed with repeat length less than that of mean of 22. Statistical significance was also observed between BPH and controls ($p = 0.019$). However, no significance was observed between CaP and BPH. We have also calculated the allelic frequencies of KLK3 polymorphism however, we could not find any association between the KLK3 gene polymorphism and risk of prostate cancer. Further, the genotypic analysis of CYP 19 [TTTA] showed slight association with genotype A2A2 being most common in cases while heterozygous A1A2 was predominant in controls as well as in BPH cases.
Conclusion: Our results suggest that AR CAG repeat polymorphism may play a relative role in prostate cancer risk. In order to confirm if KLK3 and CYP19A1 [TTTA] genotyping could be used as an additional parameter to predict PCa risk further studies are necessary on larger number of samples.

446: Persistent infection with human papilloma virus, 3q chromosomal gain and presence of circulating RNA component of the telomerase (TERC) in cervical neoplasia

[1]**Pavani Sowjanya**, [1]Jayashree Dolpady, [2]Kerstin Heselmeyer Haddad, [3]Sonia Andersson, [2]Thomas Reid, [1]Lavanya Bezawada, [4]Usha Rani, [5]Patti Garvitt, [1]Gayatri Ramakrishna

[1]Centre for DNA Fingerprinting and Diagnostics, ECIL Roadd, Nacharam, Hyderabad, AP India, [2]National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [3]Karolinska Institute, Stockholm, Sweden, [4]MNJ Cancer hospital, Hyderabad, AP, India, [5]Johns Hopkins University, Baltimore, MD, USA

While the majority of women infected with human papilloma virus show effective clearance of the virus, a subset of women show persistent infection in the cervical epithelium, which consequently progresses to neoplasia. Identification of novel biomarkers for cervix cancer progression is therefore warranted, which when used in adjunct with HPV testing will help in designing better cervical cancer screening strategies. One of the initial steps in cancer initiation is the activation of the enzyme telomerase. Previous studies have demonstrated gain of chromosome 3q in cervical cancer and in particular the sub-region 3q26 which encodes for the RNA component of the telomerase, TERC. The present investigation describes two different studies using samples from two different geographical sources to evaluate the importance of TERC and HPV in cervical carcinomas. In a pilot study, conducted with samples collected from India, we tried to evaluate if TERC-RNA can be detected in the circulating blood in women diagnosed with squamous cell carcinomas (SCC) with persistent HPV infection for its utility as a biomarker. We screened for major HPV-types prevalent in SCC samples obtained from women undergoing surgery in a city-based hospital of Hyderabad. 87% of the squamous cell carcinomas showed presence of high-risk HPV types. In the matched serum samples collected from these women we could detect circulating RNA component for TERC only in the cancer cases and not in the normal women. In a collaborative study done at National Cancer Institute, NIH, we evaluated HPV integration together with 3q chromosomal gain (by FISH) in different stages of cervical cancer progression. Cervical scrapes with TERC gain were observed in 19% of the lesions with benign histopathology, in 30% with CIN1 histopathology, in 65% of the CIN II lesions, and in 95% of the CIN III cases and 100% of the squamous carcinoma. We not only found an increase in TERC gain in correlation with the increase in the CIN grades but there also occurred a change in the HPV status from mostly an episomal form in low grades to an integrated form in the invasive cancer. In conclusion, the incremental gain of 3q26 region in progressive grades of cervical neoplasia and the detection of TERC-RNA in circulating blood is suggestive of the importance of TERC in cervical neoplasia. Studies using larger sample sizes have to be conducted to further validate the role of TERC as a biomarker in cervical screening/diagnostic strategies.

447: Genetic disorders in Arab populations reflect a remarkable diversity

Ghazi Omar Tadmouri

Centre for Arab Genomic Studies, P.O. Box 22252 Dubai, United Arab Emirates

Genetic disorders are chronic ailments that require lifelong treatment and many have no definitive cure. In the Arab World, several disorders, including chromosomal, single-gene, and multifactorial disorders are common. Some of these disorders have assumed epidemic proportions and continue to impact the health system in the region by increased rates of neonatal deaths and morbidity and mortality in children as well as in adults. The economics of genetic disorders in the Arab World is the least studied aspect of this problem and is expected to reach billions of US Dollars annually when taking into consideration only few of the most common disorders in the region. Although the expertise and resources to initiate prevention programs are available in the region, nation-wide programs to control genetic disorders are still not available in many Arab countries. It is out of this need that the Centre for Arab Genomic Disorders initiated the largest project to catalogue human genetic disorders in all Arab countries in 2004. Extensive data collection has been achieved in the United Arab Emirates, Bahrain, and Oman. Available data indicate that, in the region, more emphasis is given to clinical analyses rather than molecular analyses. More than 450 genetic disorders have been described in the Arab populations of the UAE, Bahrain, and Oman. A small fraction of these disorders are common in the three populations while a large number of genetic disorders are country-specific indicating a remarkable heterogeneity in the populations of the region. Congenital malformations and chromosomal abnormalities are the leading cause of genetic disease in the region (30–50%). Autosomal recessive inheritance is the most common mode of genetic transmission of the disease (40–55%) and could be attributed to the common practice of consanguineous marriages (35–50%). Almost of 1:4 of all genetic disorders described in the region remain with no defined molecular pathology. Many of these disorders are considered rare worldwide, but acquired higher frequencies in the region. Studying such rare diseases may illuminate genes, proteins, and pathways involved in common diseases affecting millions of people across the globe. Constructive engagement of regional efforts to build sustainable research activities based upon education and improvement of human health should be considered as an utmost priority in the Arab World.

448: Genetic factors related to ethnic variation in plasma levels of the evolutionary conserved IGF1 gene: a study evaluated towards the susceptibility of breast cancer in Mexico

[1]**Parvathi Kumara Reddy Thavanati**, [2]Aurora Escoto de Dios, [2]Alma Heidi Medina Godinez, [2]Erika Beatriz Lara Zaragoza, [3]Francisco Salazar Macias, [3]Antonio Oceguera Villanueva, [4]Kodanda Rami Reddy Kanala

[1]Instituto de Genetica, Dpto, de Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada 950, Puerta 7, Edificio P, Nivel II, Col. Independencia, Guadalajara. Jalisco, Mexico, [2]Instituto de Biologia Molecular y Medicina, Dpto, de Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada 950, Puerta 7, Edificio P, Nivel II, Col. Independencia, Guadalajara. Jalisco, Mexico, [3]Instituto de Jalisciense de Cancerologia, Hospital Civil Viejo (Fray Antonio Alcalde), Calle Hospital, Col. Centro, Guadalajara, Jalisco, Mexico, [4]Department of Anthropology, School of Biological and Earth Sciences, Sri Venkateswara University, University Road (Chandragiri Road), Tirupati, Andhra Pradesh, India

Insulin-like growth factors (IGFs) play a major key role in cell proliferation and apoptosis. IGFs are relatively stable within individuals but, substantially vary between individuals and a large component of this variation may be determined by genetic factors. Recent epidemiological studies have provided evidence that these levels are of predictive risk for several common cancers. Several polymorphisms in IGF gene have been identified, although their functional significance is not clear. We evaluated the association of the polymorphisms in IGF1, VNTR (D1S80), STR (HPRTB) and circulating levels of IGF-1 among 251 population-based control subjects enrolled in a case-control study of breast cancer. The sample consists of two Mexican populations: Mestizos and indigenous women with an age range of 20–60+ years. The total IGF-1 levels were measured using ELISA assays, and all the DNA of the subjects were genotyped for a microsatellite polymorphism in the IGF1 gene (CA 19) through Gene Scan along with VNTR (D1S80) and STR (HPRTB) using polyacrylamide gel electrophoresis. There is a variation in the plasma IGF-1 levels between Mestizos and the indigenous populations along with a difference of allelic frequency of the IGF1 gene, VNTR (D1S80) and STR (HPRTB) towards the susceptibility of breast cancer. Polymorphic variants in the IGF1 gene, and D1S80 and HPRTB polymorphisms are associated with increase in the plasma levels of IGF-1 among the two populations and the variant alleles are much more common in Mestizos than in Indigenous subjects due to genetic drift. Higher incidence of breast cancer among Mexican Mestizos population may be mediated through genetic modifiers of circulating of the IGF-1.

449: Differential gene expression profile of stomach and oral cancer in high risk region of India

[1]**Regina D. Thoudam**, [1]Dhirendra S. Yadav, [1]I. Chattopadhyay, [2]A. C. Kataki, [3]E. Zamoawia, [1]S. Kapur, [1]S. Saxena

[1]Institute of Pathology (ICMR), SJH Campus, New Delhi, India, [2]Dr. B. Borooah Cancer Institute, Gopinath Nagar, Guwahati, India, [3]Civil Hospital, Aizawl, Mizoram, India

Background: Northeastern states of India have reported significant regional differences in the incidence of oral and stomach cancers. The age adjusted rate (AAR) for oral cancer is 12.2 in Kamrup district of Assam and 57.3 for stomach cancer in Mizoram. The reason for this large variation is unknown. In the current study, gene expression profiling was done to find out if dysregulated genes due to the peculiar tobacco consumption and betel quid chewing habits, that are widely prevalent in this population, can explain the reason for such a high incidence in the occurrence of these cancers.
Material and method: RNA extracted from ten tumor tissues (five with oral squamous cell carcinoma and five with gastric adenocarcinoma) was amplified using ExpressArt AminoAllyl mRNA amplification Kit and labelled with NHS-activated Cy-3 dye. Normal tissue obtained from a site distant to tumour was used as control. The labelled probes were hybridized with Human 40K OciChip Array containing 20,106-probes. The image were analysed and quantified using Imagene. The data were normalized by locally weighted linear regression (LOWESS) method. Differential expression analysis of tumor tissue with respect to normal tissue was carried out using Genowiz™.
Results: Using stringent criteria (twofold change) 1609 and 1514 genes were differentially expressed in oral SCC (1046 upregulated and 593 downregulated) and gastric adenocarcinoma (616 upregulated and 898 down regulated) respectively. Gene Ontology revealed that genes involved in regulation of actin cytoskeleton, MAPK and Wnt signalling pathways were differentially expressed in both the cancers. Genes involved in Neuroactive ligand-receptor interaction and leukocyte transendothelial migration were differentially expressed in gastric adenocarcinoma exclusively. Genes of TGF beta signalling pathway were significantly downregulated in gastric adenocarcinoma while in oral cancer genes of T-cell-receptor and Toll like receptor pathway were significantly upregulated.
Conclusion: The genes identified in our results are of interest because of their potential roles in the natural history of oral squamous cell carcinoma and gastric adenocarcinoma. It is possible that ingestion of genotoxic chemical of tobacco and betel quid induce uncontrolled cellular proliferation through differential expression of these genes.

450: Role of genetic alterations in E-cadherin gene in gallbladder cancer and in early gallbladder epithelial lesions

[1]**Padma Priya Togarrati**, [2]VK Kapoor, [3]Vinita Agrawal, [3]Narendra Krishnani, [1]Suraksha Agrawal

[1]Sanjay Gandhi Institute of Medical Sciences, Department of Medical Genetics, Lucknow, India, [2]Sanjay Gandhi Institute of Medical Sciences, Department of Surgical Gastroenterology, Lucknow, India, [3]Sanjay Gandhi Institute of Medical Sciences, Department of Pathology, Lucknow, India

Aims and objectives: Gallbladder cancer (GBC) ranks fifth among cancers of the gastrointestinal system. Due to the high incidence rates in India, it has been described as a North Indian disease. Loss of function of E-cadherin (16q22.1) leads to increased invasiveness and metastasis in tumors. Loss of heterozygosity on 16q has been frequently detected in breast, liver, prostate, etc. The objective of the study was to investigate the role of genomic instability (MSI/LOH) in CDH1 and E-cadherin protein expression in GBC, chronic cholecystitis (CC), xanthogranulomatous cholecystitis (XGC) and normal gallbladders (GB).
Materials and methods: 40 GBC, 50 CC, 34 XGC and 15 normal GB tissues along with paired blood samples were collected. DNA was isolated from histopathologically confirmed samples by salting out method followed by PCR using fluorescently labeled primers specific for D16S421, D16S496, D16S503, D16S512, D16S2624 and

D16S3021 microsatellite markers. Denatured PCR products were run on ABI PRISM 3130 automated fragment analyzer. Immunohistochemistry was done by using mouse anti-human CDH1 (clone NCH-38) antibody.
Results: 9 (22.5%) out of 40 GBC, 7 (14%) out of 50 CC and 7 (20.6%) out of 34 XGC cases revealed MSI-H. No normal GB cases revealed MSI or LOH. A significant difference was seen between MSI-H and MSI-S among normal GBs vs. GBC groups ($p = 0.04$). 17/40 (42.5%) GBC, 9/50 (18.0%) CC and 13/34 (38.2%) XGC cases showed LOH in CDH1. LOH was significantly high in GBC vs. CC and GBC vs. normal GB groups ($p = 0.01$ and $p = 0.002$). Difference was also significantly high among XGC vs. CC and XGC vs. normal GB groups ($p = 0.04$ and $p = 0.004$). LOH at CDH1 was positively associated with the stage of the tumors ($p = 0.04$). E-cadherin expression was absent in 15/36 (41.7%) GBC, 2/46 (4.3%) CC cases and 3/33 (9.1%) XGC cases. All normal GB cases showed strong E-cadherin expression. E-cadherin expression significantly differed among GBC vs. CC, GBC vs. XGC and GBC vs. normal GB groups ($p = 0.001$, $p = 0.001$ and $p = 0.01$).
Conclusion: MSI in CDH1 may not be associated with GBC pathogenesis. Significantly high LOH in GBC vs. CC and XGC vs. CC may indicate order of severity in the progression of GBC may be GBC > XGC > CC and CC may be a precursor lesion of GBC. Absence of association of LOH in CDH1 with E-cadherin expression may warrant role of germline mutation or promoter methylation in loss of CDH1 function in GBC or some other tumor suppressor gene at 16q may have a role in GBC oncogenesis.

451: 2, 17 novel translocation in a severely oligozoospermic infertile man

[1]**S. Venkatesh**, [1]M. Kumar, [2]R. K. Sharma, [3]R. Kumar, [3]N. P. Gupta, [1]R. Dada

[1]Laboratory for Molecular Reproduction and Genetics, Departments of Anatomy, All India Institute of Medical Sciences, New Delhi, India, [2]ART centre, Army Research and Referral Hospital, New Delhi, India, [3]Department of Urology, All India Institute of Medical Sciences, New Delhi, India

Structural and numerical chromosomal aberrations are one of the most common genetic causes in male infertility. Though Y chromosomal defect attribute to impaired spermatogenesis, about 2,000 genes present on the autosomes also regulate spermatogenesis. Here we report a novel translocation involving chromosome 17 and chromosome 2 in a 34-years-old infertile male with poorly developed secondary sexual characters and severe oligozoospermia. Pedigree showed no family history of infertility. 72 h blood culture was set up, GTG banding was done, and chromosomes were classified according to ISCN guidelines. Repeated semen analysis showed highly viscous and incomplete liquefaction even after 30 min with sperm count less than 1 million/ml. Peripheral karyotype analysis revealed 46, XY t(2;17) (qter;q12) chromosome complement. To the best of our knowledge such autosomal translocation in q arm of chromosomes 2 and 17, has not been previously reported. Though several studies have been shown role of sex chromosomes in the germ cell production, autosomes also play an important role in regulation of spermatogenesis. Such autosomal translocation result in severely impaired spermatogenesis and manifest as oligozoospermia. Thus a large number of cases with autosomal structure abnormalities need to be studied to establish genotype and phenotype correlation and to understand the role of autosomal genes in germ cell development and differentiation. Additional study of case's parent genotype, fluorescent in situ hybridization and molecular screening is necessary to characterize the gene involved in translocation, position effect and its impact on regulation of germ cell development.

452: Mitotic (non-meiotic) instability of the genome is associated with early spontaneous fetal death

[1,2]**S. G. Vorsanova**, [1,2]I. Y. Iourov, [2]A. D. Kolotii, [3]E. A. Kirillova, [1]I. A. Demidova, [1]A. K. Beresheva, [1]V. S. Kravets, [2]V. V. Monakhov, [1]O. S. Kurinnaya, [2]I. V. Soloviev, [1,2]Y. B. Yurov

[1]Institute of Pediatrics and Children Surgery, Rosmedtechnologii, Taldomskaya 2, Moscow, Russia, [2]National Research Center of Mental Health, RAMS, Zagorodnoe 2, Moscow, Russia, [3]Woman's Medical Center, Moscow, Russia

Genomic instability associated with aneuploidy and polyploidy commonly causes spontaneous abortions (SA). However, the contribution of somatic genome instability to cellular/fetal death remains to be estimated. Here, we have analyzed chorionic villus samples derived from 500 consecutive SA by interphase multiprobe FISH (IMP-FISH) with DNA probes for chromosomes 1, 9, 13/21, 14/22, 15, 16, 18, X and Y. Chromosome abnormalities were found in about 50% of cases. We detected aneuploidy in 39.6% of samples (aneuploidy of chromosome 16—10%; monosomy of chromosome X—8.2%; polysomy of chromosome X—5.2%; aneuploidy of chromosome 13 or 21—5%; aneuploidy of chromosome 14 or 22—4.6%; aneuploidy of chromosome 15—3.2%; aneuploidy of chromosome 18—2.2%; aneuploidy of chromosome 9—1.2%). Polyploidy was found in 10.2% of cases. One case was a chimera with multiple sex chromosome and autosomal aneuploidy. Multiple chromosome abnormalities manifested as aneuploidy involving both autosomes and sex chromosomes was detected in 3.7% of cases. Chromosomal mosaicism (aneuploidy/polyploidy) was detected in 23.6% of all cases and 47.6% of cases with chromosome abnormalities. The survival of cells with chromosome imbalances evidences for a higher contribution of aneuploidy/polyploidy to fetal death rather than to cell death in fetal tissues. The high incidence of mosaic chromosomal imbalances indicates for a close relationship between somatic genome instability and pregnancy loss. To the best of our knowledge, the present set of SA samples is the largest one analyzed by IMP-FISH. Our data demonstrate that somatic aneuploidy and polyploidy is highly frequent in SA and provide evidences that mitotic, but not meiotic, instability of the genome is associated with early spontaneous fetal death. Supported by Philip Morris USA Inc.

453: HSD11 regulates the sensitivity of HeLa cells to multiple apoptotic stimuli through Bcl-xL

[1]**Fei Wang**, [1]Wei Wu, [1]Congli Cai, [1]Ning Liu, [1]Shiying Miao, [2]Shudong Zong, [1]Linfang Wang

[1]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Peking Union Medical College, Tsinghua University, 5 Dongdan Santiao, Beijing, China, [2]National Research Institute for Family Planning, WHO Collaboration Center for Research in Human Reproduction, 12 Da Hui Si, Beijing, China

HSD11 was cloned from human testis cDNA library by our group; the HGNC approved Symbol for this gene is C15orf23 (alias TRAF4AF1, TNF receptor associated factor 4 associated factor 1). TRAF family members are critical factors involved with apoptosis, proliferation and differentiation processes by the linkage of the receptors to signaling molecules along different cellular pathways. Treatment of

overexpressed HSD11 sensitized Hela cells with TNF-α stimulus and other studies revealed that ectopically expressed HSD11 also rendered cells sensitive to ultraviolet light. In addition, C15orf23 knock-down by RNA interference resulted in cells resistant to multiple apoptotic stimuli, showing that HSD11 is capable of influencing both the death receptor and the mitochondrial pathways. It is known that Bcl-2 family members are critical to both apoptotic pathways that the expression levels of Bcl-2, Bcl-xL, Bax and Bak are examined in overexpressed HSD11 and C15orf23 knock-down cells. We found that overexpression of HSD11 could down-regulate Bcl-xL protein level; however, C15orf23 knock-down could maintain the expression level of Bcl-xL in the present of apoptotic stimuli. Thus the present preliminary data provide evidence that HSD11 may modulate the sensitivity of Hela cells to multiple apoptotic stimuli by regulating the expression level of Bcl-xL.

454: Induction of tumorigenesis by APLP2 through regulation of cell survival and cell apoptosis

[1]**Wei Wu**, [1]Fei Wang, [1]Xiaofei Wei, [1]Yongqiang Tian, [2]Shudong Zong, [1]Shiying Miao, [1]Linfang Wang

[1]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Peking Union Medical College, Tsinghua University, 5 Dongdan Santiao, Beijing, China, [2]National Research Institute for Family Planning, WHO Collaboration Center for Research in Human Reproduction, 12 Da Hui Si, Beijing, China

APLP2 is a member of a multigene family that includes APP and APLP1. Although a number of activities have been attributed to the APP family, an overall function has not been definitively established. The intracellular domain of APP can regulate the expression of EGFR, thereby be involved in EGFR-mediated tumorigenesis. Recently we showed that APLP2 is a G-protein coupled receptor that binds the specific ligand rMIS. The resulting complex activates the ERK signal pathway and promotes cell proliferation. In the present study, we showed that the interaction between rMIS and APLP2 blocked cell apoptosis induced by CHX, including changes of the apoptotic molecules, such as p53, bcl-2, caspase-3, and p21. We also found that both protein and mRNA levels of APLP2 were significantly higher in pancreatic tumor cell lines than in normal pancreatic tissue; i.e., the positive ratios of APLP2 protein in pancreatic tumor cell lines were significantly greater than in normal pancreatic tissue. Hence, we proposed that APLP2 might be an important factor involved in the induction of tumorigenesis by regulating cell survival and cell apoptosis.

455: Codon 72 of TP53 gene polymorphism in oral cancer and stomach cancer in high-risk region of India

[1]**D. S. Yadav**, [1]R. D. Thoudam, [2]A. C. Kataki, [3]E. Zamoawia, [4]Y. Verma, [1]S. Kapur, [1]S. Saxena

[1]Institute of Pathology (ICMR), SJH Campus, New Delhi, India, [2]Dr. B. Borooah Cancer Institute, Guwahati, Assam, India, [3]Aizawl Civil Hospital, Aizawl, Mizoram, India, [4]S.T.N.M. Hospital, Gangtok, Sikkim, India

Background: North Eastern states of India have reported a very high incidence of tobacco associated cancers. Highest age adjusted incident rate of oral cancer (12.2) had been reported in Kamrup district of Assam and stomach cancer (57.3) in Aizawl. The allele constitution at codon 72 of the tumour suppressor gene TP53 plays a major role in inducing apoptosis in TP53 mutant cells. This gene contains single nucleotide polymorphism that encodes either arginine (Arg) or proline (Pro) at amino acid codon 72 of the p53 protein. The relationship between human cancer susceptibility and arid TP53 polymorphism at codon 72 is controversial. In current study we have investigated the association between this polymorphism and patients with stomach and oral cancer.

Material and method: In the current study, we investigated TP53 codon 72 polymorphism using proline or arginine specific primers from the peripheral blood cells (PBC) representing constitutional DNA from 112 patients with stomach cancer and 100 patients with oral cancer and 207 controls. Cases and controls were matched for their origin, sex and age. PCR-RFLP method was performed for detection of polymorphic genotypes among cases and controls.

Results: The frequencies of genotypes Arg/Arg, Arg/Pro and Pro/Pro were 19.6, 67.8 and 12.5%, for stomach cancer cases, 27, 57 and 16% for oral cancer cases respectively and 21.7, 55 and 23% for controls. The frequency of pro/pro ($p = 0.0233$) genotype is higher in controls as compared to stomach cancer cases and the frequency of pro/Arg ($p = 0.0270$) genotype is higher in stomach cancer cases as compared to control.

Conclusion: Our data suggest that pro/pro genotype works as a protective factor in gastric cancer. Whereas pro/arg genotype increases the risk of gastric cancer. In our study we find no significant association of codon 72 polymorphism of TP53 gene and oral cancer susceptibility.

456: Cooperation between EZH2, NSPc1-mediated histone H2A ubiquitination and Dnmt1 in HOX gene silencing

Jiangang Yuan, Li Zhao, Yuangang You, Xudong Wu

National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, National Human Genome Center, 5 Dong Dan San Tiao, Beijing 100005, China, China

An intricate interplay between DNA methylation and polycomb-mediated gene silencing has been highlighted recently. Here we provided evidence that Nervous System Polycomb 1 (NSPc1), a BMI1 homologous polycomb protein, plays important roles in promoting H2A ubiquitination and cooperates with DNA methylation in HOX gene silencing. We showed that NSPc1 stimulates H2A ubiquitination in vivo and in vitro through direct interaction with both RING2 and H2A. RT-PCR analysis revealed that loss of NSPc1, EZH2 or DNA methyltransferase 1 (Dnmt1), or inhibition of DNA methylation in HeLa cells de-represses the expression of HOXA7. Chromatin immunoprecipitation (ChIP) assays demonstrated that NSPc1, EZH2 and Dnmt1 bind to the promoter of HOXA7, which is frequently hypermethylated in tumors. Knockdown of NSPc1 results in significant reduction of H2A ubiquitination and DNA demethylation as well as Dnmt1 dissociation in the HOXA7 promoter. Meanwhile Dnmt1 deficiency affects NSPc1 recruitment and H2A ubiquitination, whereas on both cases EZH2-mediated H3K27 trimethylation remains unaffected. When EZH2 was depleted, however, NSPc1 and Dnmt1 enrichment was abolished concomitant with local reduction of H3K27 trimethylation, H2A ubiquitination and DNA methylation. Taken together, our findings indicated that NSPc1-mediated H2A ubiquitination and DNA methylation, both being directed by EZH2, are interdependent in long-term target gene silencing within cancer cells.

457: Somatic genome instability closely associates with cerebellar neurodegeneration in the ataxia telangiectasia

[1,2]**Y. B. Yurov**, [1,2]I. Y. Iourov, [1,2]S. G. Vorsanova, [3]T. Liehr, [2]A. D. Kolotii, [2]A. K. Beresheva, [2]I. A. Demidova, [2]V. S. Kravets, [1]M. K. Tagirova, [2]O. S. Kurinnaya, [1]V. V. Monakhov, [1]I. V. Soloviev

[1]National Research Center of Mental Health, RAMS, Zagorodnoe 2, Moscow, Russia, [2]Institute of Pediatrics and Children Surgery, Rosmedtechnologii, Taldomskaya 2, Moscow, Russia, [3]Institute of Human Genetics and Anthropology, D-07740, Jena, Germany

Ataxia-telangiectasia (AT) is an early onset neurological disease caused by mutations in a single gene, ATM (ataxia telangiectasia mutated). ATM deficiency affects many organs, but the hallmark of AT is progressive cerebellar neurodegeneration. AT is associated with prominent chromosomal instability in developing immune system cells, reflecting failures of somatic recombination by mechanism of V(D)J joining. The balanced rearrangements involving breakpoints on chromosomes 14 and 7 in lymphocytes are the most remarkable laboratory finding in AT. Chromosomal instability and the possibility of altered somatic recombination in cells of neural system in AT remain unexplored. Here we monitored interphase chromosome breaks in neural cells of the AT brain. We found that neural cells in the cerebellum and in the cerebrum (frontal cortex) of the AT brain characterized by two to fivefold increase of mosaic aneuploidy randomly affecting different chromosomes in a stochastic manner. Therefore, mosaic neural aneuploidy is a prominent genetic feature of the cortex and the cerebellum in the AT brain. Cerebellar cells were additionally featured by a dramatic 5- to 20-fold increase of unresolved interphase chromosome breaks and aneuploidy involving specifically chromosome 14, 7 and X in an age-dependent manner. Thus, the maintenance of neural genome stability is markedly affected in the degenerating cerebellum. The persistence and propagation of non-random chromosome breaks in neural cells of the AT cerebellum indicate that neural genome may undergoes somatic rearrangements similarly to V(D)J-associated recombination in the immune system cells. In summary, two complementary forms of genomic instability (non-balanced chromosome breaks and aneuploidy) are the possible aetiological factors that underline neurodegeneration in AT. This work was supported by AT Children's Project, USA (RUB1-1618-MO-06).

458: Mutations in acute myeloid leukemia-1 gene result in down regulation of RUNX2 and LAT gene expression

Jyoti Zack, Daman Saluja

Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, India

Acute myeloid leukemia -1 (AML-1/PEBP2A2/Runx1), a transcription factor is essential for the hematopoiesis and is one of the common targets of chromosomal translocation. Its family includes RUNX2, which is essential for the osteoblast differentiation and RUNX3 a protein essential for the antiproliferation and apoptosis of gastric epithelium, for differentiation of certain root ganglion nerve cells, and for the establishment of mature CD8+ cytotoxic T- lymphocytes. CBFbeta heterodimerizes with Runx1 and mediates the DNA binding through Runt domain. Mutations in the Runt domain of AML-1 protein have been shown to be associated with leukemia. To understand the role of mutant Runx-1 in the progression of leukemia, we studied altered expression of AML-1 regulated genes. Initially, using Electrophoretic mobility shift assay (EMSA), DNA binding activity of wt AML and mutant AML-1 was compared. The expression of RUNX3 gene (AML-2) is regulated by the AML1 protein and vice versa. It has also been reported that AML-1 also regulates the linker for activation of T cells (LAT). AML1 and its mutants were cloned in bacterial as well as mammalian expression vector. The recombinant AML1 protein expressed in Xli-Blue cells was partially purified using Ni-NTA affinity column. The probe1 (52bp DNA) specific to AML-2 promoter and probe2 (36bp DNA) specific to LAT promoter were body labeled using αP32-dATP by Polymerase chain reaction. The gel purified αP32-dATP labeled probe was used to study its interaction with the wild type AML1 and its mutants expressed in mammalian cells as well as purified protein expressed in bacterial cells. While wild type AML1 binds to both the probes (AML-2 and LAT specific probe), mutant AML (K83E) failed to bind with the labeled probes. In contrast, R174Q mutant shows low binding with the DNA sequence. The DNA protein interaction was specific as unlabelled probe could compete with the binding while nonspecific had no effect on DNA-AML interaction. The structure of mutant AML1 proteins was predicted by homology modeling using InsightII software. Using AMBER8.0 software, the molecular dynamics also shows that the binding of protein to the DNA gets distorted because of the mutations found in the AML1. The effect of mutant AML1 protein on the ex-vivo expression of RUNX2 and LAT will be shown by RT-PCR. Thus, our results suggest that mutations in RUNX1 gene (AML-1) result in loss of expression of AML-1 regulated genes thereby resulting in loss of hematopoietic differentiation.

459: Genotype–phenotype correlations for CNVs identified in patients with mental retardation; increased array resolution refines detected CNVs, selects candidate genes and diagnoses known syndromes

[1]**Farah R. Zahir**, [1]Shelin Adam, [1]Linlea Armstrong, [2]Allen D. Delaney, [3]Patrice Eydoux, [2]Sylvie Langlois, [1]Hong Qian, [1]Margot Van Allen, [2]Marco A. Marra, [1]Jan M. Friedman

[1]Department of Medical Genetics, University of British Columbia, 4500 Oak Street, Box 153, Vancouver, BC, V6H 3N1, Canada, [2]British Columbia Cancer Agency Genome Sciences Center, 675 West 10th Avenue, Vancouver, BC, V5Z 1L3, Canada, [3]Children's and Women's Health Center of British Columbia, 4480 Oak Street, Vancouver, BC, V6H 3N1, Canada

Submicroscopic copy number variants (CNVs) identified by array genome hybridization (AGH) are now known to cause mental retardation (MR) as frequently as cytogenetically detectable genetic defects. We screened 100 trios each comprising a child with idiopathic MR and both normal parents using Affymetrix 500K GeneChip® Human mapping assays and detected causative de novo CNVs in 16 cases. Two patients carried overlapping deletions of 9p11.2p13.3; one a ~11Mb deletion from 33.7 to 44.7 Mb, and the other a ~4.6Mb deletion from 34.1 to 38.7 Mb. The distal non-overlapping portion (from 38.7 to 44.7 Mb) is highly enriched in low copy repeat (LCR) elements and poorly assayed by Affymetrix 500K SNP probes. We re-screened both patients using Affymetrix 6.0 GeneChips® which have >threefold higher coverage of the region and include non-SNP probes which show more robust performance for regions involved in LCR. On this platform we found the single deletion in the first case to be composed of two smaller deletions (a ~6Mb deletion from 33.4 to 39.3 Mb and a 0.7 Mb deletion from 44.1 to 44.8 Mb) as well as an additional small deletion (of 0.6 Mb from 44.2 to 44.8 Mb) in the second patient previously undetected. Choosing the appropriate platform is important when assaying genomic regions involved in complex genomic architecture.

We found a ~10.8 Mb deletion of 7p15.3 in another patient. Our patient's CNV overlaps with five other deletion and duplication CNVs reported in the DECIPHER database, suggesting the identification of a novel region that is prone to disease causing variation. A 6.9 Mb duplication of 8q12.1 in another case involves the CHD7 gene, which causes CHARGE syndrome when deleted. This patient's phenotypes include defects in organ systems (cardiac, ocular, auditory and genital) affected in CHARGE syndrome. In another patient we identified a rare whole chromosome uni-parental disomy (UPD) for chromosome 16 that we believe is pathogenic. This heterodisomic UPD would not have been detected by a non-SNP probe AGH platform. We also screened patients who were normal on Affymetrix 100K GeneChip® AGH with the higher resolution 500K platform and detected causative CNVs in two patients. A 4p16.3 deletion in a patient subsequently diagnosed with mild Wolf-Hirschorn syndrome and a novel 1.5 Mb duplication of 8q22.3 in another case. These data highlight the usefulness of higher resolution AGH and demonstrate the varying benefits of different probe sets.

460: Co-transcription and intergenic splicing in vertebrates

Changqing Zeng, Guanting Lv, Weihua Chen

Beijing Institute of Genomics, the Chinese Academy of Sciences, Beijing Airport Industrial Zone B6, China

The transcription of a protein-coding gene usually begins from the promoter and ends at the termination site of the same gene in a regulated manner. However, transcription may also read through the intergenic region and stopped at various sites of the downstream gene that consequently generated a fused transcript with exons of two neighboring genes. Evidence also showed that some fused transcripts could be spliced and translated into chimeric proteins. To learn if this phenomenon of co-transcription is a rare situation of adapted transcription error or another mechanism of one gene for multiple products, over 8 million human and 2 million mouse ESTs and full-length cDNAs were aligned to their refseqs respectively. In total 582 human and 198 mouse co-transcripts were identified. Thus far we validated 23 (51%) and 18 (40%) fused transcripts out of 45 randomly chosen co-transcripts from the two species by RT-PCR and protein identification. Interestingly, about half of these co-transcripts displayed tissue-specific expressions. Although the intergenic distance was contributive to co-transcription to certain extent, signals of the polyadenylation and the 5′ splicing site of the last exon in upstream gene appeared not to directly relate to the generation of co-transcripts. Furthermore, fused mRNAs could be classified as five major patterns according to the exons involved in intergenic splicing. The most dominant splicing occurs between the second exon from the last ($n - 1$) of the upstream gene and the first exon ($+1$) of the downstream one, leading to the elimination of the translation stop codon of the first gene to generate translatable chimeric transcripts. Moreover, proteins residing in one multi-protein complex or sharing one metabolic or signal transduction pathway tended to produce co- transcripts, indicating possibly more efficient and cooperative regulation for their functions. These results indicate that co-transcription and intergenic splicing are widely spread perhaps as a specific mechanism to increase the complexity of gene expression in vertebrates.