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ABSTRACTS

Single gene disorders

075: Prenatal and Postnatal diagnosis for Beta thalassemia and sickle cell anemia

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The current study aims to summarize the data obtained from screening of families with probands on regular transfusions and carriers having elevated Hemoglobin A2 levels for Beta Thalassemia and Sickle cell anemia using 155 cases for Postnatal diagnosis and 34 for Prenatal diagnosis. Molecular analysis was done using PCR followed by Reverse Dot blot analysis and sequencing. Of the 34 cases for Prenatal diagnosis, 9 were found to be normal for RDB and sequencing, 15 were heterozygous for one of the seven common mutations, 8 were homozygous mutant and 2 were compound heterozygous. VNTR analysis using 16 microsatellite markers were done to rule out maternal contamination. Of the 155 cases (includes probands and parents) for which molecular testing was done showed 40 were normal, 82 heterozygous and 33 homozygous for one of the 7 common mutation by RDB. IVS1-5(G-C) being the most common,as reported earlier in the descending the order of IVS1-5 (G-C), FS41/42, HbS, FS 8/9, Codon 15, HbE and 619 bp deletion. We present here a unique case referred to our centre, a couple who came to our clinic at 12 weeks of her gestation. The father showed elevated levels of Hemoglobin A2 in HPLC indicative of the presence of a hemoglobin variant, which was suspected to be HbE. Proband was not alive and so there was no information of the mutations in that family. RDB showed a normal picture in both the parents (including HbE) as well as in the Chorion villus sample. On sequencing both the parents showed FS Codon5 (-CT) mutation and normal sequence in fetus. FS Codon 5 (-CT) is a rare mutation which accounts for less than 1% of mutations in India. In addition, the father was heterozygous for Hemoglobin D Iran, which led to a similar picture to HbE. The mother was advised to continue her pregnancy. This highlights the need to increase awareness among public regarding the importance of genetic testing in proband so that prenatal diagnosis can be done in subsequent pregnancies.

076: CC2D1A is involved in autosomal recessive non-syndromic mental retardation in a Pakistani Family

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Mental retardation is the most frequent handicap among children and young adults affecting 1–3% of the general population. To date of 11 loci, with linkage to nonsyndromic autosomal recessive mental retardation (NSARMR), only four genes have been found with associated mutations. The MRT3 locus was initially mapped in families with NSARMR to an interval of 2.4 Mb and is caused by deletion of CC2D1A gene. We have studied a three generation Pakistani family with three affected individuals. The affected individuals of the family segregate NSMR and have psychomotor developmental delay in childhood. None of the diseased individual reveals the presence of autism spectrum disorders or seizures. The DNA samples from five family members, including three affected females were used for genotyping of microsatellite markers linked to known MRT (MRT1-11) loci. Analysis of the results indicates linkage of the studied family to MRT3 (19p13.12) locus with a maximum two-point LOD score of 2.08 at markers D19S892 and D19S556. The same markers yielded multipoint LOD score of 2.64. Sequencing of CC2D1A gene is in progress to identify the mutation responsible for NSARMR in Pakistani family.

077: Molecular analysis in pediatric Wilson's disease: 13 novel mutations

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Objectives: WD (Wilson's disease) is an autosomally inherited defect characterized by the accumulation of copper in liver, brain and other vital organs. This occurs primarily due to mutations in the P-type ATP7B gene for the trans-Golgi membrane protein resulting in the accumulation of copper. The main aim of our study was to identify common mutations causing WD in pediatric patients of Asian-Indian origin.
Methods: Fifty nine pediatric patients from 52 unrelated families were enrolled for this study after obtaining written informed consent.

Diagnosis of WD was based on mandatory clinical and biochemical analysis. Mutational screening was carried out using a combination of Single Strand Conformation Polymorphism (SSCP) and Conformation Sensitive Gel Electrophoresis (CSGE). Sequencing was performed for samples showing mobility shift. Screening was done for all the exons (including the flanking splice sites) as well as the promoter region. Detected sequence changes were also looked for in 60 unrelated controls.
Results: Causative mutations were identified in 49 out of 59 (83.05%) samples studied. None of these mutations were detected in controls. A total of 24 different mutations were found, including 13 missense, 3 nonsense, 2 splice site, 5 deletions and 1 insertion mutation. Thirteen (54.1%) of the detected mutations were novel. Maximum numbers of mutations were identified in exon 2 followed by exon 14.
Conclusion: Commonly reported mutations H1069Q and R778L (from the Caucasian as well as the Asian non-Indian population) were not detected in this study and a very heterogeneous spectrum of mutations emerged. Our preliminary study indicates that there is a possibility that exons 2 (p.Q271X) and 14 (p.G1061E) mutations maybe very common mutations in the Indian population but this assumption has to be supported by large multicentric studies (which would be representative of the ethnic heterogeneity of the Indian population) before arriving to a definite conclusion.

078: Modifier gene mutations in Beta thalassemia

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Beta thalassemia is the most prevalent monogenic hemoglobin disorder and causes significant mortality. It is also characterized by wide variation in phenotypes, underscoring the contribution of modifier genes. Excess unpaired α-chain is responsible for much of the dyserythropoesis and hemolysis in β-thalassemia and determinants, which reduce the amount of free α-chain, reduce the severity. Hence knowledge of α-globin gene mutations and deletional β-globin cluster mutations, which elevate the level of HbF in adult stage, are essential to evaluate β-intermedia cases. We present the molecular details of these determinants found in patients of eastern part of India and Bangladesh.

Deletional α-globin mutations are detected by standard method and are present at frequencies of 16, 5 and 12.5% for 3.7, 4.2 kb and SA deletion respectively in α-thalassemia patients. SEA and a new deletion KOL have also been detected. Among non deletional α-globin mutations the polyadenylation (poly A) signal mutation AAT AAA>AATA–, Hb Sun Prairie and Koya Dora are found in appreciable numbers. Using PCR-based assays designed for easy screening of patients, the polyadenylation mutation is detected very frequently in our population. Out of 77 putative α-thalassemia patients, nine carried the poly A signal mutation and four were homozygotes; the allele frequency was 8.5%. Out of 13 β-thalassemia intermedia patients, three were positive for this mutation. Inheritance of certain cis or trans acting mutations of β-globin cluster increases the ability of adult erythroblasts to synthesize γ-globin chains. Several deletions and point mutations can precipitate the condition in a pancellular way, and the value of HbF may get raised above 90%. We studied 18 probands with raised HbF, ranging between 9.2 and 97.2%. Of these, 17 chromosomes bearing the Asian Indian inversion-deletion have been identified, from nine families. Three persons from two families had HPFH-3 and one person had the Asian deletion. The clinical profile of the entire patient group mentioned above will be presented. Study of heterocellular or swiss type HPFH revealed that the Senegal haplotype and the XmnI RFLP is linked to high HbF phenotype but significance did not reach the desired 0.05 level ($p > 0.063$).

079: Not so simple! Hemophilia B as a quasi-quantitative condition with mutations showing variable penetrance

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Hemophilia B (MIM# 306900), an X-linked bleeding disorder caused by mutations in human coagulation factor IX, can be classified as severe, moderately severe and mild based on the plasma levels of factor IX among affected individuals with respect to normal factor IX activity assayed in the patients' plasma (<1, 2–5, 6–30%, respectively). Recently, we identified hemophilia B to be a disease with mutations showing clinical variation. Here, we have analyzed the differences in sequence and structural properties among identical mutations with varying phenotypes (IMVPs) by comparing with mutations with uniform phenotypes (MUPs), with recurring reports in Haemophilia B mutation database derived based on clinical cut-offs. IMVPs ($n = 51$) occur in less conserved mutant sites with more tolerated substitutions compared to MUPs ($n = 100$), though no significant differences were observed in structural properties. A preponderance of CpG site mutations and Arg as the mutated residue in IMVPs compared to Cys in MUPs was observed. Hence, a CpG site substitution at less conserved Arg site might have an increased propensity of expressing variable phenotypes. Further, we considered clotting activity as continuous phenotype and investigated the variation expressed by these missense mutations. We derived mean deviation about the geometric mean and classified the mutations by unsupervised learning into three clusters viz., cluster 1 ($n = 11$), 2 ($n = 33$) and 3 ($n = 157$) with centroids 9.17, 4.00 and 0.45, respectively. As the allelic spectrum encompasses mutations that show least phenotypic variation as well as those showing phenotypic plasticity, we establish hemophilia B as a quasi-quantitative condition characterized by variable penetrance, relocating it from the section of simple monogenic diseases into a space that marks the conceptual continuum of Mendelian to complex diseases. Mutations showing phenotypic plasticity were characterized by relatively less conserved mutant sites and more tolerated conservative substitutions. Based on this study and available literature we speculate that modifier genes at other loci, epigenetic interactions and environment may serve individually or cumulatively to bring about the phenotypic heterogeneity. Certain mutations have been consistently reported to cause mild phenotype, calling for a check in indiscriminate termination of fetuses with such mutations following prenatal diagnosis.

080: Mutational analysis of PMP22, MPZ, GJB1 and EGR2 genes in Charcot-Marie-Tooth Neuropathy (type1) patients from Bashkortostan Republic of Russia

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Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy (HMSN) is a clinically and genetically heterogeneous disorder of peripheral nervous system. The HMSN frequency in Bashkortostan Republic (BR) is 10.3:100000. HMSN type I is prevalent

in Bashkortostan. We examined CMT1A duplication of 17p11.2-p12, mutations of PMP22, MPZ (P0), GJB1 (Cx32) and EGR2 genes in 131 unrelated patients. Based on electrophysiological criteria, 67 of 131 patients were diagnosed as CMT1, 9 patients—as CMT2, and 55 patients had not electrophysiological data. The CMT1A duplication was found in 26.7% of 131 CMT patients. In PMP22 gene we determined one nucleotide change—c.309-29 G>A, which haven't been described previously. In the families without PMP22 duplications, we revealed Ser88Ley (C263G) mutation in one patient (0.8%), and synonymous nucleotide change 714C>T (Ser238Ser) in two patients (1.6%) in the MPZ gene. We detected 4 missence-mutations in the GJB1 gene in 18 of 131 unrelated patients (13.7%), including 1 novel mutations:Pro87Ala (c.259C>G), found at a frequency of 10% among HMSN patients, Arg22Gln (c.65G>A)—2.98%, Arg15Gln (c.44G>A)—1.5% and Thr86Ile (c.257C>T)—0.8%. The frequent mutation Pro87Ala was revealed in patients of Bashkir ethnic origin. It was linked to one and the same haplotype that evidences about founder effect. No changes were revealed in EGR2 gene in HMSN patients. Thus, specific spectrum of mutations was detected in HMSN patients from Bashkortostan that contributes to development of optimal DNA diagnostics for this region.

081: Novel mutations in FRMD7 in Russian families with X-linked congenital motor nystagmus

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X-linked congenital motor nystagmus is a common inherited oculomotor disorder characterized by repetitive uncontrollable ocular oscillations, unassociated with a number of ocular or neurological diseases with onset typically at birth. The loci for X-linked CMN have been mapped to Xp11.3-p11.4 and Xq26-q27 (NYS1). Three more loci have been described for autosomal-recessive and autosomal-dominant form of CMN without any gene identification. The molecular characterization of NYS1 has been solved by Tarpey et al., who identified mutations in FRMD7, a gene with un-known function. The aim of our research was searching for FRMD7 mutations in three Russian families, who had already shown the linkage with Xq26-q27 locus, and 26 sporadic unrelated cases with X-linked CMN. Sequencing analysis of all exons and intron-exon junctions of FRMD7 was performed in 29 affected individuals. We identified five novel, previously unreported mutations (three missense, one nonsense and a small deletion) in FRMD7: c.47T>C (Phe16Ser), c.804G>T (Trp268Cys), c.657G>T (Lys219Asn), c.1524G>A (Trp508Stop), c.1492delT, and one known mutation c.902A>G (Tyr301Cys). The mutations c.47T>C (Phe16-Ser), c.1524G>A (Trp508Stop) and c.1492delT were identified in all three familial cases; the mutations c.804G>T (Trp268Cys), c.657G>T (Lys219Asn) and c.902A>G (Tyr301Cys) were observed in four sporadic cases (the mutation c.657G>T (Lys219Asn) was identified in two unrelated patients). Thus the results of our study confirm the role of FRMD7 in the pathogenesis of X-linked CMN.

082: Molecular screening approach for identification of mutations causing familial hypercholesterolaemia in Western Australia

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It is well established that elevated plasma concentrations of low density lipoprotein (LDL) cholesterol are associated with increased risk of cardiovascular disease. Autosomal dominant familial hypercholesterolaemia (FH) is one of the most common human diseases caused by mutations in a single gene, affecting some 10 million people worldwide. FH is characterised by high levels of circulating LDL caused by reduced clearance of LDL from plasma by the LDL-receptor pathway, leading to premature coronary heart disease (CHD). The majority of FH is caused by mutations in the LDL-receptor (LDLR) gene; over 800 have been described. Of the estimated 40,000 cases of FH in Australia, 80% are undiagnosed and less than 10% are being adequately treated. Treatment with statins is highly effective in lowering plasma LDL-cholesterol. The FH Western Australia (FHWA) pilot program has been established to identify and treat individuals with FH in WA, by cascade screening from index cases. A genetic screening protocol has been designed that consists of three stages: (1) detection of 20 commonly occurring mutations by an amplification refractory mutation system (ARMS), (2) detection of deletions/duplications by multiplex ligation-dependent probe amplification (MLPA) and (3) exon-by-exon sequencing of the LDLR gene. Using this approach, a mutation was identified in 36 of 50 consecutive index patients enrolled in FHWA. Eleven patients carried mutations that were detected by ARMS, and six carried mutations detected by MLPA, indicating that almost half of FH index patients carrying a mutation could be identified without the need for exon-by-exon sequencing. In summary, we present a cost-effective genetic screening strategy for FH and preliminary data for the FHWA pilot program. DNA test results will be useful for cascade screening from FH index cases, leading to early detection of other previously undiagnosed family members and enabling early intervention and prevention of premature CHD and death.

083: Prenatal diagnosis of single gene disorders: 10 years experience from a referral center

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In the absence of treatment for most life limiting single gene disorders, families opt for prenatal diagnosis (PND) and selective termination of affected fetuses for preventing birth of affected babies. Direct mutation testing and linkage analysis (in the absence of known mutations) can be used to track the affected allele(s). We present our experience of PND of single gene disorders in last 10 years. In most cases fetal tissue used was chorionic villus biopsy. The most common referral was for prenatal diagnosis of β thalassemia and other hemoglobinopathies for which we have offered PND in 915 at risk pregnancies using amplification refractory mutation system (ARMS) or linkage (6.71%) and were able to give confirmed results in 97.5% of cases. About 20% fetuses were affected. Spinal Muscular atrophy (SMA) is probably the second most common autosomal recessive (AR) single gene disorder after thalassemia in India. We have performed prenatal testing for SMA (looking for homozygous deletion of exon 7 of SMN

gene) in 125 families out of which 32 fetuses (25.5%) were affected. Another AR disorder which was thought to be rare in India is Cystic fibrosis (CF). We were able to delineate a very different mutational spectrum including novel mutations in CF patients and offer PND to 29 families out of which 6 fetuses were affected. Common X linked disorders referred for PND included Hemophilia A/B and Duchenne Muscular dystrophy (DMD). For Hemophilia (A and B) about 100 families were offered PND, mostly by linkage and 31 babies were affected. For DMD 176 PND were done (multiplex PCR and linkage) detecting 37 affected babies. Megalencephalic leukodystrophy, probably the commonest cause of Leukodystrophy in Indians is caused by mutation, c.135_136insC. We have tested about 25 families and offered PND in three families. PND for some rare disorders has also been offered. We do not have follow up of the pregnancy outcome for all tested fetuses but available data shows almost 100% accuracy. We feel that there is a felt need for PND amongst at risk families and there should be more centers equipped with molecular diagnostic and prenatal diagnostic facilities in our country.

084: Mutational analysis of ATP7B gene and genotype-phenotype correlation in Wilson's disease patients from the Volga-Ural region of Russia

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Wilson disease (WD) is an autosomal recessive disorder of copper transport, caused by mutations in the ATP7B gene (13q14.3-q21.1) encoding a copper-transporting P-type ATPase. Defects in ATP7B lead to copper storage in different organs and tissues, principially in the liver and in the brain. The disease manifests as progressive liver degeneration and neurological impairment, frequently with kidney malfunction. The clinical symptoms and age at onset of Wilson disease are highly variable. The purposes of this research were the detection of disease-causing mutations and analysis of correlation between phenotype and genotype. We examined 43 Wilson's disease patients from 32 WD families of various origins from the Volga-Ural region of Russia (Tatars, Russians, Bashkirs and mixed origins). The mutational screening of ATP7B was performed by SSCP-analysis and sequencing of shifted exons. We have identified three novel mutations (p.A718P, p.L1057P, p.K1315_R1316delinsE) and six mutations that had been previously described (c.2298_2299insC, p.E1064K, p.H1069Q, c.3400delC, c.3559+1G>T, p.L1305P). The most common mutation, p.H1069Q, was observed in 46.88% of the WD chromosomes. The frequency of this mutation in Russians was 50.00%, Bashkirs—44.4%, Tatars—40.9%. The patients homozygous for the H1069Q mutation had more frequently neurologic presentations and a later age of symptoms onset than H1069Q heterozygotes and patients without the H1069Q mutation. One of the novel mutations, p.K1315_R1316delinsE, was found with an relatively high frequency in Bashkirs (22.2%) and Tatars (18.1%). One patient was homozygous for p.K1315_R1316delinsE mutation and five patients were heterozygotes. The patients with p.K1315_R1316delinsE had more severe clinics with early manifestation and hard liver disorders. Two Russian patients were heterozygous carriers of the novel mutation p.L1305P and one Russian patient was the p.A718P heterozygote. The mutation p.E1064K was determined mainly in Russians—in 15% of the WD chromosomes. The remaining mutations were detected rare and limited to one or two patients. This study has expanded our knowledge on the spectrum of mutations in the WD gene in patients from Volga-Ural region of Russia and has found correlation between type of mutation and clinical manifestations.

085: Primary Immunodeficiency disease database: A discovery tool for the genomics research

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Resource of Asian Primary Immunodeficiency diseases (RAPID) is a web-based compendium of molecular alterations in primary immunodeficiency diseases. The main objective of this database is to provide detailed and user-friendly information of genes and proteins involved in primary immunodeficiency diseases along with other pertinent information about signaling pathways, protein-protein interactions, mouse studies and their gene expression profile in different cells of the immune system. All this information is well organized and connected such that RAPID should serve as a discovery tool for prediction of candidate primary immunodeficiency disease genes, a useful feature for immunologists, physicians and researchers. RAPID also hosts mutation viewer tool for the visualization of mapped sequence variations. Using bioinformatics analysis, all primary immunodeficiency disease genes have been mapped to immune signaling pathways from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, interaction networks from Human Protein Reference Database (HPRD), microarray expression profiles across different study from Gene Expression Omnibus (GEO), Array Express, Stanford Microarray Database (SMD) and also mapped to mouse studies from Mouse Genome Informatics (MGI) for the representation of allele based phenotypes and anatomical systems affected for the available PID genes. With such varied data available so far for all the known primary immunodeficiency disease genes, RAPID would also serve as a connecting link between the phenotype and the genotype.

086: DNA-diagnostics of choroideremia in Russian family

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Choroideremia is a congenital X-linked ocular disease characterized by the degeneration of the choriocapillaris, the retinal pigment epithelium and the photoreceptor of the eye. The disorder leads to the progressive loss of vision beginning at an early age resulting from the complete atrophy of the choroid and retina. The CHM gene responsible for choroideremia, is located on Xq21.2, contains 15 exons and encodes a protein, the Rab escort protein-1 (REP1), which is involved in membrane trafficking. Currently, there are about 110

mutations in CHM gene, and these are all nonsense, frameshift or splice site mutations leading to choroideremia. The purpose of our study was elaboration of DNA-diagnostics of choroideremia in affected Russian patient. Sequencing analysis of all exons and intron-exon junctions of CHM in affected man showed a previously described nonsense mutation Arg253Stop (c.757C>T) in exon 6 of CHM. Restriction analysis performed in a sister of the patient, who has small clinical presentations of choroideremia, detected mutation Arg253Stop (c.757C>T) in heterozygous state. Thus the given DNA-analysis results revealed a disease-causing mutation Arg253Stop (c.757C>T) and confirm the diagnosis 'choroideremia' in the clinical case.

087: Molecular genetics of primary microcephaly in Indian families

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Microcephaly (small head) is defined as a condition in which the head circumference of an affected individual is >3 SD below the population age and sex related mean. The small cranial capacity results from underlying hypoplasia of the cerebral cortex rather than abnormal development of the overlying skull. Primary microcephaly (MCPH; OMIM 251200) is a distinct subtype that is defined by the absence of associated malformations and of secondary or environmental causes. It is inherited as an autosomal recessive trait. Patients with MCPH have mild to severe mental retardation but without any other neurological deficits. It has an incidence of 1/30,000–1/250,000 live births in western populations. The actual incidence of MCPH is not known in India, but it could be higher in south Indian states of Karnataka, Andhra Pradesh, Kerala and Tamil Nadu where ~33% of marriages are consanguineous. It is a genetically heterogeneous disorder with six known loci: MCPH1-MCPH6. So far, genes for MCPH1 (MCPH1), MCPH3 (CDK5RAP2), MCPH5 (ASPM) and MCPH6 (CENPJ) loci have been identified. We have ascertained a total of 42 families with MCPH from the states of Karnataka, Andhra Pradesh and Tamil Nadu. We have carried out linkage analysis of a majority of these families using PCR-based microsatellite markers from the candidate regions of six known MCPH loci. Our analysis showed that the ASPM gene is a major cause of MCPH in Indian families. The presence of unlinked families to any of the known MCPH loci in our dataset suggested the involvement of a seventh locus for this disorder. We are performing a genome-wide screening of unlinked families to identify a novel MCPH locus. DNA sequence analysis in MCPH5-linked families has identified one known and four novel mutations in the ASPM gene in a homozygous state.

088: X-linked Adrenoleukodystrophy gene polymorphisms and mutation in Indian population

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Objectives: X-linked adrenoleukodystrophy (X-ALD) is the most frequent inherited peroxisomal neurodegenerative disorder caused by impairment in peroxisomal β-oxidation of very long chain fatty acids (VLCFAs) due to mutation in ABCD1 gene which encodes a peroxisomal ABC half-transporter (ALDP) involved in import of VLCFA from cytoplasm to peroxisomes. The study aims at establishing genetic association of ABCD1 and ABCD2 gene polymorphism with various phenotypes of X-ALD and identify and classify mutations.
Methods: Nineteen adrenoleukodystrophy patients and 70 unrelated matched healthy subjects (controls) were enrolled. Patients were recruited on the basis of MRI and/or VLCFA analysis. Blood was collected, genomic DNA isolated, and PCR for ABCD1 and ABCD2 genes performed using exon flanking region primers to sequence the exonic region of the genes by ABI 3100 automated sequencer. Results: We identified a total of six single nucleotide polymorphisms (SNPs) in controls and adrenoleukodystrophy patients in both the genes. Five SNPs include 5′ UTR polymorphism C/T at position-59 (frequency 16.67%) and exonic polymorphism G/A at position 1548 in sixth exon (frequency 18.6%). Two intronic (IVS-1) polymorphisms G/A were identified at position-105 (frequency 5.4%) and C/T at position-16 (frequency 5.4%) and another novel unique polymorphism G/T at position-51 in IVS3. One novel polymorphism was also detected C/A in the ABCD2 gene at position-59 (frequency 23.18%) in IVS4. Additionally, we have identified two mutations in patient sample, one in exon at position 796, resulting in change of amino-acid glycine to arginine at position 266 in protein. Another mutation at 3′ splice site in IVS4 is at position-2. It is denovo as it is not present in the proband mother. SNP results analysis does not show any significant association with any of the six polymorphisms in various phenotypes of adrenoleukodystrophy.
Conclusion: ABCD1 and ABCD2 gene polymorphisms alone may not be important risk factors for adrenoleukodystrophy. More genes like ABCD3 and ABCD4 may be involved in this complex disease process whose protein products may interact with the ALDP. The mutation in splice site in ABCD1 is very interesting. We expected formation of higher molecular weight cDNA due to splice site mutation. However, there was no formation of cDNA of the ABCD1 gene, indicating the aberrant or incomplete splicing which may result in absence or translation of a defective protein.

089: Application of human genome knowledge in a tertiary ophthalmic Institution in India

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Purpose: To determine the application of human genome knowledge in diagnosis, inheritance and gene mapping in a tertiary ophthalmic institution in India.
Methods: Microsatellite markers for RB1 gene and X chromosome and whole genome homozygosity mapping using the Affymetrix 10K

XbaI Gene Chip and ExcludeAR program were performed on four different families with three different mendelian diseases—retinoblastoma, congenital cataract and Leber congenital amaurosis. The coding exons of the RB1, NHS and lebercilin genes were sequenced to screen for mutations co-segregating with the phenotype.
Results: Genomic knowledge helped us to (1) track the defective RB1 allele in two different autosomal dominant retinoblastoma families; in one family the male neonate did not carry the allele, whereas another unborn child, carried the defective allele and in a two year intense follow-up showed signs of the tumor; (2) refine clinical diagnosis of a congenital cataract family to Nance-Horan syndrome and this was possible with the lab data on this family with linkage to Xp22.13 and identifying a truncating mutation C115T in exon 1 of NHS gene, resulting in conversion of glutamine to stop codon (Q39X); (3) map the gene in the third family, this was a second-cousin marriage—there were two girls who were affected with LCA. In this family analysis revealed two homozygous regions in both the children for which the parents were heterozygous—6q12-q16.3 and 7q21.11-q22.3. A 24.5cM region with 139 consecutive SNPs at 6q12-q16.3 had a homozygous novel c.955G>A missense mutation in lebercilin in the last base of exon 6, causing disruption of the splice donor site. This mutation ablates the correct splice donor site, leading to mis-splicing at an alternative donor site 5 bp into the adjacent intron, which results in a 5 bp insert in the transcript. This in turn leads to a frameshift and premature truncation of the protein.
Conclusion: In these four families, genomic knowledge helped us not only to identify the causative genes but also to help these families in genetic counseling. These studies had benefited the family and it also emphasizes the importance of bench to bedside practice.

090: Simple and cost-effective approach for quantitative detection of heterozygous deletion genotypes in diagnosis of DMD/BMD carriers

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Deletions involving whole single or multi-exons of the DMD gene account for more than half of all patients with Duchenne/Becker muscular dystrophy (DMD/BMD). A number of techniques are currently used to identify these mutations routinely. However, the methods for detecting carriers of deletion or duplication genotypes either require sophisticated equipment or involve laborious protocols or expensive kits. Some methods are also susceptible to false deletion positive results arising from single base deletion or polymorphism affecting ligation or primer binding site. We compare two dosage methods for detecting heterozygous deletions within the gene mutation hot-spots in our population. Quantitative real-time assays were designed covering exons 6, 8, 45 and 50. Reference exon plasmid constructs were cloned and used to generate standard curves for validation of amplification efficiencies. Melt curve analysis was used to rule out non-specific PCR products. Gene dosage analysis showed that 79.9% of all deletion patients can be screened using this four exons assay set while addition of another three exons, i.e. 3, 20 and 47, would cover all common gene deletions in our population. We also carried out analysis using multiplex ligation-dependent probe amplification (MLPA) which covers all 79 exons. Concordant results were obtained except for one case of false positive deletion of an extra exon shown by MLPA screening. Both approaches were useful for carrier screening in our families. Out of a total of 156 affected families, 71 were due to deletions of single or multi-exons. Carrier mothers were detected in 47 families (66.2%), of which 12 (16.9%) were obligate carriers. Our quantitative real-time exon-specific assay sets are rapid, reliable and cost-effective for detecting heterozygous carriers of deletion genotypes. It will also be useful for other ethnic populations with similar common DMD deletion mutation profiles.

091: Novel CUL7 mutation in 49 Yakut patients with short stature syndrome

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Yakuts have a high frequency of some hereditary diseases, because they have experienced a serious bottleneck effect. Hereditary short stature syndrome is one of the major concerns in Yakuts. We have identified 49 patients with short stature syndrome in 43 Yakut families with pre- and post-natal non-progressive growth failure, facial dysmorphism and normal intelligence. The average of birth length was 42.0 cm $\pm$ -6.2 standard deviation score (SDS), and that of weight was 2.330 kg. A genome-wide linkage analysis for these families revealed linkage to region 6p21.1 with the highest multipoint LOD score of 24.6 at D6S282. We applied a homozygosity mapping approach and narrowed the causative gene to the same locus of the 3-M syndrome and the gloomy face syndrome (Huber et al., 2005). We found a novel homozygous 4582insT mutation in CUL7, which resulted in a frameshift and subsequent premature stop codon at 1553 (Q1553X). The clinical presentations of 49 patients with short stature syndrome (from birth to 45 years) in Yakut were similar to those of 3-M syndrome. However, they have a high frequency of neonatal respiratory distress (41.98%) and a low frequency of X-ray abnormalities. These findings may provide better understanding of the clinical diversity of short stature syndrome with the CUL7 mutation.

092: SCA-LSVD: A new locus specific disease database (LSDB) for cataloging variations associated with Spinocerebellar ataxias

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Spinocerebellar ataxias (SCAs) are a group of neurodegenerative disorders clinically and genetically heterogeneous, characterized by overlapping clinical symptoms among subtypes that include progressive gait ataxia, dysarthria, pyramidal and extrapyramidal signs peripheral neuropathy, ophthalmoplegia. Nearly 30 loci have been identified to be associated with ataxia which includes both candidate linkage regions as well as well characterized genes. Repeat expan-

sions in 10 of the characterized loci have been associated with SCA. Due to a wide clinical spectrum of SCAs and overlapping features, the clinical classification becomes difficult in the absence of genetic analysis. Therefore, an understanding of Phenotype to Genotype correlations in SCAs would be integral in clinical diagnosis, and planning optimal treatment/disease management strategies. As a step towards this we have created a new locus specific variation databases for SCAs (SCA-LSVD) aims to catalog and integrate information on SCAs from all over the world. Currently, the database houses detailed information on the clinical features like age at onset, symptom at onset, mode of inheritance, and besides repeat length also harbors information on SNP variations, genotypes and haplotypes associated with the affected individuals from more than 450 families across India. Amongst the genetically characterized loci only five of them has so far been observed to be associated with SCA in India accounting for nearly 40% of the SCAs. Amongst these, SCA2 has the highest frequency (33%) followed by SCA12 (30%), SCA1 (21%), SCA3 (10%), SCA7 (2%). Preliminary Genotype-Phenotype correlations of the ataxias reveal subtle features that distinguish SCA subtypes with overlapping cerebellar features. For instance, individuals of SCA12 have a characteristic tremor not observed in other ataxias and is specific to an ethnic population. Once a close link with tremor, ethnicity and SCA12 has been established, it is much easier to clinically classify SCA12. This would be a very useful starting point for understanding the molecular correlates of phenotypes in ataxia which is a multi locus disease where related molecular mechanisms converge to overlapping phenotypes. SCA-LSVD would also be an important resource to identify novel candidate loci for SCAs. This database would be soon made available online and would allow submission and viewing of variants associated with SCA.

093: Heterozygous deletion of the 3′ region of *TYR* is one of the potential mutations which remains uncharacterized in 15% of the OCA1 patients worldwide

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Oculocutaneous albinism type 1 (OCA1) is an autosomal recessive hypopigmentary disorder, associated with common developmental anomalies of eye. Currently, almost 200 mutations of Tyrosinase gene (*TYR*, 11q14.2) are known that results in pathogenic conditions of OCA1, but in 15% of the patients, the second mutation has never been identified. We recently proposed that partial deletion of 3′-region of *TYR*, which has ~98.55% sequence identity with a known pseudogene (*TYRL*, 11p11.2-cen), could represent such 'unidentified mutations' (UCMs); since commonly used PCR-based mutation screening would not detect heterozygous gene deletion (Prog Retin Eye Res 26: 323–358, 2007). Therefore, we examined our OCA1 patient pool for heterozygous deletion of the 3′-region of *TYR*. We studied two unrelated OCA patients in which, the second mutation in *TYR* could not be identified despite screening the entire coding sequence, splice junctions and promoter region of the gene. Using limited amount of genomic DNA from these two samples, we amplified the 3′-region of *TYR* by semi-quantitative PCR with fluorescent-labeled primers, followed by assessment of the gene products via genemapper software (Applied Biosystems). The results suggested heterozygous deletion of *TYR* in one patient, previously identified as a heterozygote for Arg278stop (R278X) at *TYR* locus. A SNP based assay is currently being implemented to corroborate our finding. A positive result would support our hypothesis that heterozygous gene deletion represents at least some of the refractory mutations in *TYR*, currently known as 'UCMs' in 15% of the OCA1 cases worldwide. Also, we propose that the patients lacking the second *TYR* mutations should be screened for heterozygous gene deletion. This study is supported by funds from Council of Scientific and Industrial Research (CSIR), Govt. of India.

094: Mitochondrial gene mutations as a cause of non-syndromic hearing impairment among probands from Andhra Pradesh

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Hearing impairment affecting 1 in 1,000 newborns is a highly heterogeneous disorder with majority of the cases being non-syndromic (NSHI). It can result from a mutation in a single gene or from a combination of mutations in different genes or by environmental factors and also by interaction between genetic and environmental factors. Mitochondrial DNA mutations have been found to be associated with both aminoglycoside induced hearing impairment and NSHI. The common mtDNA mutations causing NSHI include A7445G, T7510C, T7511C in tRNA Ser (UCN) gene and A1555G in 12S rRNA gene. This mutation is more frequent in many populations and is considered as a primary factor underlying the development of deafness. However, the expression of the deafness phenotype associated with this mutation requires contributions of nuclear modifier genes or aminoglycoside antibiotics which affect the phenotypic manifestation by enhancing or suppressing the biochemical effect of the mutation. A nuclear modifier gene on chromosome 8 is thought to account for deafness in those individuals who do not have aminoglycoside exposure. 484 probands with profound bilateral sensorineural hearing impairment were ascertained for various epidemiological parameters and 303 DNA samples of patients including 25 families and 200 control samples were screened for mitochondrial mutations by PCR–RFLP followed by genotyping on 2% agarose gels stained with ethidium bromide. The epidemiological results revealed: preponderance of males (58.7%) as compared to females (41.3%) indicating high risk for hearing impairment in males. High incidence of positive family history (59.9%), parental consanguinity (58.0%) and delayed milestones (59.5%) was recorded in males as compared to females. 32.6% of the cases with delayed milestones were considered as due to vestibular dysfunction. Screening for mutations revealed absence of A7445G, T7510C and T7511C in tRNA Ser (UCN) gene. Three homozygous mutants were found for A1555G mutation in 12S rRNA gene. All the three probands had profound non-syndromic hearing impairment and had no history of exposure to aminoglycoside antibiotics suggesting the possible involvement of nuclear modifier gene that might interact with the mutated 12SrRNA and affect the phenotypic manifestation by enhancing or suppressing the biochemical effect of the mutation. None of the controls showed the mutation. This is the first study from India showing the prevalence of A1555G mutation among non-syndromic hearing impaired patients.

095: Seven new mutations have been found in STX11, PRF1 and UNC13D genes in a group of Russian FHL patients

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FHL is a rare recessive disorder of immune regulatory pathways. X-linked lymphoproliferative syndrome (XLP) caused by EBV may be pathologically indistinguishable from FHL. FHL-causing mutations have been found in PRF1 (10q22), UNC13D (17q25.1) and STX11 (6q24.1) genes. These genes code proteins involved in the lymphocyte cytotoxity. XLP-causing mutations have been found in SH2D1A (Xq) and XIAP (Xq) genes. We have investigated a group consisted of 18 unrelated Russian patients. The disease manifestation age ranged from a few days to 9 years. DNA samples have been investigated for mutation in PRF1, UNC13D and STX11 genes coding areas by direct sequencing. In the cases when mutations have not been detected in these genes and X-linked inheritance could not be ruled out we have investigated SH2D1A gene. Investigation of UNC13D gene has detected 6 compound heterozygote patients, carring two mutations, 1 homozygote patient carrying one mutation and one patient carrying only 1 mutation in heterozygote state. Four frame shift mutations have been detected: c.627delT on one chromosome, c.1828insA on two chromosomes (new), c.2346_2349delGGAG on three chromosomes and c.3037insG on five chromosomes (new) in UNC13D gene. A new mutation c.2216_2239del on one chromosome, one previously reported splicing mutation c.322-1G>A on one chromosome and one new possible missense mutation c.3173T>C (p.1058Leu>Pro) on one chromosome have been also found in UNC13D gene. Two mutations have been detected with higher frequency (c.2346_2349delGGAG on three chromosomes and c.3037insG on five chromosomes) so we propose that there might be 'hot points' in UNC13D gene. One patient has been found to carry a new mutation (c.675_679del/c.675_679del) in STX11 gene in homozygote state. Nucleotide substitutions have been detected on 3 chromosomes in PRF1 gene. One new nonsense mutation c.1283G>A (p.428TRp>Stop) and two possible new missence mutations c.1000G>A (p.334Gly>Ser) and c.916G>T (p.306Gly>Cys) have been found. Among patients carrying no mutations in cording area of PRF1, STX11 and UNC13D genes one hemizygote patient carrying mutation c.164G>T (p.55Arg>Leu) CD01961 in SH2D1A gene have been detected. Genetic lesions have been detected in over 60% of FHL patients: eight patients (44%) carried mutations in UNC13D gene, two patients (11%) carried mutations in PRF1 gene and one patient (6%) carried mutation in STX11 gene. According to these results it is possible to consider UNC13D to be the first candidate gene in the group of Russian FHL patients.

096: Genetic polymorphism and expression analysis of Glutathione *S*-Transferases in breast cancer patients

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Recent reports have revealed the increased incidence of breast cancers with industrialization and urbanization in many countries, including India. Glutathione *S*-transferases (GSTs) are a large family of proteins that participate in antioxidant defenses and xenobiotic detoxification. They occur both in cytosol and microsomes. Eipdemiological studies have revealed that individuals with null genotypes for GSTM1 and GSTT1 are associated with many adverse health effects, including risk of developing cancer of bladder, colon, lung, skin and stomach. The studies clearly demonstrate that lack of expression of certain isoforms of GSTs make the individuals susceptible for cancers induced by toxic chemicals. In the present study we examined the possible correlation of GST gene polymorphism and breast cancer incidence using data from breast cancer patients undergoing treatment at Indo-American cancer hospital and MNJ cancer hospital in Hyderabad, India. Also studies were conducted on the pattern of changes in GSTs in cancerous and adjacent non-cancerous tissues obtained from breast cancer patients undergoing surgery. DNA was extracted from blood samples and genotyped for GSTM1, GSTT1 and GSTP1 gene deletion using multiplex PCR. These studies revealed higher frequency of GSTM1 gene deletion in breast cancer patients (38%; $n = 36$) compared to normal individuals (26%; $n = 15$), suggesting a possible correlation of GST M1 gene deletions and breast cancer risk. Further studies on the expression of GSTs revealed a significantly higher expression of GST Pi with no appreciable changes in GST alphaand GST Mu tissues ($n = 24$) compared to the corresponding normal tissue ($n = 24$). Along with the elevation of GST-Pi levels, high molecular weight proteins (470 KDa) cross reacting with GST antibodies were detected only in surgically desected tumor biopsies but not in non-cancerous tissues adjacent to the tumor. Based on MALDI-TOF analysis, the high molecular weight band were identified as synaptotagmin V bound to GST-M1 with 47% sequence coverage after processing on an MS-FIT search engine. In conclusion, our studies reveal an association between GST M1 gene deletion and breast cancer risk and over expression of GSTPi in cancer tissues. Further studies are needed to understand the functional role of GST-synaptotagmin complex in human breast cancers.

097: Identification of disease causing mutations in Familial Hypertrophic Cardiomyopathy patients from Andhra Pradesh

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Familial Hypertrophic Cardiomyopathy (FHC) is an autosomal dominant inherited disorder of the cardiac sarcomere characterized by left ventricular hypertrophy and myocyte and myofibrillar disarray with variable clinical and morphologic expression. It is the most common cause of sudden death in otherwise healthy young athletes. Gene association studies and linkage analysis have led to the identification of at least 12 genes responsible for the development of FHC. It is important to establish genetic and clinical status of patients in India. We have investigated disease causing mutations selectively in MYH7 and MYBPC3 genes, which are frequently involved in FHC in the western population. To date, we have identified six mutations, one in the MYH7 gene and five in the MYBPC3 gene, including one novel splice site mutation in 19th intron of the MYBPC3 gene. The p.R787H mutation in

the MYH7 gene was identified in four families though it could be a sporadic mutation in two families. We have analyzed the clinical presentation of 12 members from the four families harboring the p.R787H mutation and the results revealed a wide clinical heterogeneity. There does not appear to be a significant correlation of the clinical heterogeneity with age, gender and angiotensin converting enzyme (ACE) gene polymorphism. We have carried out sequence and structural analyses of the mutant MYH7 protein, in order to understand the molecular basis for the pathogenic affect of the p.R787H mutation. Our results indicate that the Arginine to Histidine mutation may affect the binding of the myosin heavy chain to the myosin essential light chain thereby compromising muscle contraction. We also identified the p.C1123ter mutation in the MYBPC3 gene in a 3-year-old child. The mother and elder female sibling of the proband also harbored the mutation but were asymptomatic, indicating a possible role of modifier gene(s) such as ACE. It is our endeavor to delineate disease causing mutations in FHC patients in order to develop molecular diagnostic strategies and to carry out genotype phenotype correlation analysis, which will help clinicians to provide better patient management and genetic counseling.

098: Molecular heterogeneity of Beta-thalassaemia and associated disorders in multiethnic setting of Southern Bengal, India

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Introduction: Beta thalassaemia is one of the inherited genetic disorders caused by mutations of the Beta-globin gene and poses a major health and socio-economic burden in the Mediterranean region, Middle-east, Indian subcontinent, Burma and in South–east Asia. India presents an alarming picture with respect to the frequency distribution of the diseased phenotypes as well as the carriers. The double heterozygous condition like E-Beta-thalassaemia is also the commonest type of thalassaemia in eastern part of India, Burma and Southeast Asia.
Objectives: The Anthropological Survey of India, in view of severity and the limited scope for management of the disease, has initiated a community genetic approach through mass awareness and massive population-screening program as the preventive measure. The objective of the present study is to identify the Beta-Thalassaemia mutations and other abnormalities in multiethnic setting of southern Bengal.
Methods: More than 5,000 unrelated individuals aged 13–30 years from the coastal South 24-Parganas district of West Bengal have participated in the mass screening drive for beta thalassaemia under the Community Genetics Extension Program. The methods used for the present study were CBC, HPLC, ARMS-PCR and sequencing of beta-globin gene. The haplotype patterns of the mutations were also detected by RFLP.
Results and Conclusion: The label of awareness in the studied area was very poor. Altogether 13.7% of the subjects were identified to carry abnormalities of hemoglobin in one or other forms. The IVSI-nt5-G>C was found to account for about 75% of all the mutations, followed by cd41/42 (-TCTT), cd15 (G>A) and others. In high-risk zones like eastern India this prospective premarital screening would be an ideal approach for preventing haemoglobinopathies and thalassaemic births.

099: Molecular characterization of oculocutaneous albinism Type 1 (OCA1) mutations found in Indian population

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Oculocutaneous albinism (OCA), which results in congenital hypopigmentation of ocular and cutaneous tissues, is reported as one of the four major causes of childhood blindness in India. Tyrosinase (TYR) being the key enzyme in the first two rate-limiting steps of melanin (pigment) biosynthesis, we selected it as the first candidate to investigate the molecular basis of OCA in the 50 Indian pedigrees. We detected 4 reported and 8 novel pathogenic changes in TYR and the results suggested that $\sim 50\%$ of OCA patients harbour TYR mutations (i.e. OCA1 cases) as a result of founder effect. To decipher the molecular basis of OCA1 pathogenesis, the missense mutations were introduced in normal TYR cDNA clone by site-directed mutagenesis, expressed in HEK293 cells and and their enzymatic activities (both tyrosine-hydroxylase and DOPA-oxidase) were measured. Biochemical assays revealed that, compared to the wildtype protein, all the mutants were almost devoid of any enzymatic activity. However, western blot analysis detected similar protein levels in wildtype as well as mutants, except two, where the mutant proteins were drastically reduced; mRNA expression studies are underway with these two mutations. Next, to explore the processing defect of TYR mutants at the subcellular level affecting enzymatic activity of the protein, immunohistochemical studies were carried out with appropriate ER and Golgi markers; which suggested ER retention of three TYR mutants. Currently immunoprecipitation and Endoglycosidase H digestion are being pursued to confirm our result. This study is supported by funds from Council of Scientific and Industrial Research (CSIR), Govt. of India.

100: NPHS2 and WT1 mutations in Indian children with steroid-resistant nephrotic syndrome

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Introduction: Steroid resistant nephrotic syndrome (SRNS) is a heterogeneous disease, characterized by heavy proteinuria and renal failure. Mutations of NPHS2 and WT1 genes, lead to early onset of heavy proteinuria and rapid progression to end-stage renal disease. In this study, we investigated the prevalence of NPHS2 and WT1 mutation among Indians and their association with clinical outcomes
Method: For this study we enrolled 40 children with sporadic SRNS and 40 steroid sensitive nephritic syndrome subjects as control. Renal biopsy in SRNS patients revealed mesangial proliferative glomerulonephritis ($n = 8$), focal segmental glomerulosclerosis (FSGS; $n = 16$), and minimal change disease ($n = 16$). The mean age of onset of SRNS was 4.65 years. Mutation analysis was performed by conformation sensitive gel electrophoresis of all exons of NPHS2 and WT1 gene. PCR products were screened for sequence alteration by CSGE. If an aberrant band was detected by CSGE, the corresponding PCR product was sequenced. Common NPHS2 variant R229Q was detected by restriction digestion of exon 5 PCR product with ClaI restriction enzyme.
Result: Mutations were identified in 6 (15%) patients. We found compound heterozygous mutation R229Q and R322P in NPHS2 in one patient with MesPGN. Three patients with FSGS were found to have R229Q in heterozygous condition. One patient with FSGS was heterozygous for 4 bp deletion TAAT in exon 5 of NPHS2 gene leading to frame shift. R322P and 4 bp deletion are novel changes. One female patient with FSGS had IVS9+4C>T in WT1 gene and karyotyping revealed complete sex reversal with 46, XY genotype.

Out of these six patients three responded to tacrolimus and three responded to cyclosporine.
Conclusion: In this ongoing study we have detected mutations in significant number of patients. Our study demonstrated that mutation in NPHS2 and WT1 are also present in Indian patients with SRNS.

101: Study of the gene causing congenital insensitivity to pain among Israeli-beduins

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Background: Congenital insensitivity to pain (CIP) syndrome is one of the rare hereditary sensory autonomic neuropathies. We characterized a Beduin family from the Negev, in which three individuals suffer from this disease.
Aim: To identify the mutation which causes the disease.
Methods: A SNP genotyping using the GeneChip mapping 250K array was performed with samples from the three affected individuals and one unaffected parent in the family. Homozygosity mapping and sorting of genomic regions were performed with software specially programmed for this purpose. Several regions were selected upon their physical size and number of SNPs they contain. These regions were genotyped for all members of the family with microsatellite markers and linkage was found to one region. Sequencing of the genes in the interval was done to identify the mutation causing the disease.
Results and Conclusions: We have mapped the locus to a 14.2Mb region on chromosome 2q. Screening of candidate genes in this region identified a protein-damaging mutation in SCN9A gene, which encodes for the voltage-gated sodium channel Nav1.7. This mutation was not found in 130 healthy Beduins.

102: Mutation analysis in Indian patients with x-linked adrenoleukodystrophy

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Introduction: X-ALD is the most frequent peroxisomal disorder. It is caused by mutations in ABCD1 gene. Its symptoms include deterioration in school performance, behavioral disturbances, impaired vision and hearing, dementia, speech difficulty and urinary incontinence. Diagnosis of ALD is suspected by clinical, radiological and biochemical findings (high plasma VLCFA levels). Mutation analysis is the only method for confirmation of ALD. Data on mutation studies from India are lacking.
Objectives: To do mutation analysis of Indian ALD patients.
Methods: 2ml of blood was taken in heparin tube (for VLCFA analysis) and 5ml of EDTA blood (for mutation analysis) from the patients who were clinically and radiologically suspected of ALD. Eleven Patients who have high plasma VLCFA levels were enrolled and subjected for mutation analysis. Primers were specifically selected to avoid amplification of fragments of the 3′ partial pseudogene and all the 10 exons comprising the coding region of ABCD1 gene were amplified using polymerase chain reaction. Conformation sensitive gel electrohoresis (CSGE) was standardized for mutation screening. Finally, sequencing was done in Patients found positive by CSGE.
Results: Out of 11 patients, mutations were found in seven patients. Three novel mutations (67_83del17 in exon 1, IVS4-2A>G in intron 4, 1939_40insGG in exon 9) and three known mutations (1202G>A in exon 3, 1547T>C in exon 6, 1679C>T in exon 7) were found. One of the mutations (1679C>T) was found in two patients.
Conclusion: ABCD1 gene mutation analysis helps in correct diagnosis of ALD, facilitates prenatal diagnosis, carrier detection and ensures proper genetic counseling.

103: Genetic analysis of families with autosomal recessive retinal degenerations

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Hereditary retinal degenerations or dystrophies are a group of disorders characterized by inherited, progressive degeneration and death of retinal tissue. They are clinically and genetically heterogeneous and primarily involve degeneration of retinal photoreceptors. Retinitis pigmentosa is one of the major forms of retinal degeneration with at least 50 genes identified so far in affected families. In order to conduct a screen of known disease loci in families with recessive RP, we used an approach of screening for homozygosity at candidate gene loci in affected individuals. 34 families with autosomal recessive RP (ARRP) and related disorders were included in the study of which 25 families were consanguineous. Patients and family members were clinically evaluated and blood samples were collected for DNA extraction after obtaining consent. Microsatellite markers flanking 23 known candidate genes for retinal dystrophy were genotyped in available members of all families. Families in which homozygosity was detected in all affected members at a given gene locus were further screened for mutations in the relevant gene. Coding regions of the genes were amplified using exon-specific primers and subjected to direct sequencing. Screening of 23 gene loci revealed homozygosity in affected individuals of 10/34 families. The loci are at chromosomes 6p21.3, 1p22.1, 1p31, 4p16.3, 1q31, 4p12, 15q26, 8q11, 14q11, 1q31, 10q23. Mutation screening in the relevant candidate genes revealed homozygous mutations that are possibly pathogenic. This approach relies on the presence of consanguinity and enabled a rapid preliminary screen of known loci in a genetically heterogeneous disorder.

104: Origin of Friedreich's ataxia (FRDA) mutation predates divergence of Indian and Caucasian populations

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Friedreich's ataxia (FRDA) is an autosomal inherited recessive ataxia caused by expansion of GAA repeats in the intron 1 of frataxin gene.

The GAA repeats polymorphic in the normal individuals are restricted to a threshold which varies in length from 7 to 16. Once unstable these repeats expand from 120 to over thousand repeats in the affected individuals. Haplotype analysis has revealed that expanded alleles arise through an intermediate premutation stage from large normal alleles (LNs) of GAA repeat (>12) and the frequency of the LNs determine prevalence of FRDA in a population. In this study, we have undertaken a detailed haplotype analysis of 23 FRDA families which had tested positive for homozygous GAA expansion and 200 ethnically matched normal individuals of Indian origin. The families though inhabitants of diverse geographical regions were all of Indo-European (I.E) origin. An earlier study by the Indian Genome Variation Consortium has revealed that I.E populations are genetically closer to CEU in HAPMAP. Therefore, we undertook this analysis using tag SNPs derived from CEU population. These SNPs span a 200 kb region encompassing the GAA repeat and also include two SNPs (ITR3-rs3829062; FAD1-rs11145465) which have earlier been associated with GAA expanded alleles in the French population as well as few Indian families of eastern India. We observed a significant association ($p < 10\text{-}6$) of four SNPs rs3829062 (93%), rs11145326 (93%), rs7861997 (79%) and rs11145465 (73%) with the expanded alleles. The SNPs rs3829062 and rs11145465 have been significantly associated with expanded alleles in French population with frequencies of 100 and 90%, respectively. These results suggest that Indian and French population share a common founder which predates divergence of these populations. Compared to 17% frequency of LNs in French population we observed its frequency to be 4.8% in the Indian population. This correlates with differences in FRDA prevalence between the two populations. The haplotype associated with the LNs in Indian population is also shared with the expanded alleles. It would be interesting to determine whether the expanded alleles have arisen from de novo recurrent mutations of LNs in the Indian population or through admixture with the CEU population.

105: SNP based genome wide homozygosity mapping in consanguineous families with autosomal recessive retinitis pigmentosa: Identification of a novel loci/ mutations

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Purpose: Retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) are, respectively, the commonest and the most severe retinal dystrophies causing blindness. Over 40 genes in RP and 13 genes in LCA, have been implicated. Only affecteds from three consanguineous families, affected with severe childhood onset retinal dystrophy were studied to identify the disease causing genes.
Methods: A thorough ophthalmic examination that included electroretinogram, fundus photograph was carried out on affected and unaffected family members and homozygosity mapping was performed using the Affymetrix 10K Xba1 GeneChip and ExcludeAR program. Based on the homozygosity results the coding exons of the lebercilin and CERKL genes were sequenced to screen for mutations co-segregating with the phenotype.
Results: Family I had two affected children, but the second child aged 11 months died due to asphyxia. Analysis using EXCLUDE AR spread sheet program identified a significant 24.5cM homozygous region at 6q12-q16.3, both the affecteds had a homozygous novel c.955G>A missense lebercilin (LCA5) mutation in the last base of exon 6, causing disruption of the splice donor site. This mutation ablates the correct splice donor site, leading to mis-splicing at an alternative donor site 5 bp into the adjacent intron, which results in a 5 bp insert in the transcript. This in turn leads to a frameshift and premature truncation of the lebercilin protein. Family II also with two affecteds had only one significant 55.5cM homozygous region identified at 2q24-2q33, overlapping the previously characterized RP26 locus. The gene implicated in RP26-Retinitis pigmentosa, CERKL, did not show any mutation. The affected consanguineous family III had 5 members affected with recessive retinitis pigmentosa. Homozygosity analysis revealed a 30 cM significant homozygous region at 6p21.1-12.3 which has not been reported so far.
Conclusions: Genome wide homozygosity mapping using oligonucleatide SNP arrays is a simple, rapid method to locate genes underlying recessively inherited disease in consanguineous families. In this way a novel lebercilin mutation was identified in an LCA family. The respiratory related pathology in the deceased child could be the result of a defect in the primary cilia. More significantly two new loci harboring retinal dystrophy genes which have not been reported so far have been identified.

106: Genotype analysis in a multi-racial population: Co-inheritance of α-thalassaemia with heterozygous β-thalassaemia in Malaysia

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Thalassaemia is a public health problem in Malaysia where 4.5% of Malaysian Chinese are α0-thalassaemia carriers and about 3.5–4.5% of the population are carriers for β-thalassaemia. Individuals with double heterozygosity for α- and β-thalassaemia and heterozygous β-thalassaemia show a similar haematological picture. Co-inheritance of α- and β-thalassaemia in both partners may result in pregnancies with either Hb Bart's hydrops foetalis or β-thalassaemia major, or pregnancies with both disorders. Therefore, a study on the co-inheritance of α-thalassaemia in 322 β-thalassaemia carriers (Chinese, Malay, Indian and indigenous population) was carried out. The frequency of α-thalassaemia in the β-thalassaemia carriers was 12.7% (41/322), with a carrier frequency of 7.8% for the SEA deletion, 3.7% for the α3.7 deletion, 0.9% for Hb Constant Spring and 0.3% for the α4.2 deletion. Double heterozygosity for α- and β-thalassaemia was confirmed in five out of the 41 couples and the risk of Hb Bart's hydrops foetalis was confirmed in two of these couples. Detection of the SEA deletion in the Malaysian Malays in this study confirms that Hb Bart's hydrops foetalis can occur in this ethnic group. Results from this study have provided new information on the frequency and different types of α-thalassaemia in Malaysian β-thalassaemia carriers.

107: Mutations in HBB gene in thalassemia patients from Bangladesh and correlating them with phenotypic severity of thalassemia

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Thalassemia is one of the most prevalent hemoglobinopathies in Bangladesh. A recent study shows that about 4.1% of Bangladesh population are carriers of β-thalassemia trait and affected β-thalassemia birth per thousand is 0.106. However, there is no systemic information on the genetic background of the disease in Bangladesh. In an attempt to reveal different β-globin mutations associated with β-thalassemia as well as to find a correlation between different mutations and phenotypic severity in Bangladesh population, we screened significant number of thalassemic patients in molecular level to find the underlying genetic mutations. We amplified and sequenced the genomic region considered to be most prone to mutations in thalassemic patients throughout the world population. Our study has revealed several mutations. Some of these corroborate with findings made on other populations. However, some mutations appear to be novel.