Abstracts

KS – Keynote Speeches

LS – Lunch Seminar

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S2 – Symposium on "Developmental Genetics & Genomics"
S3 – Symposium on "Cancer Genetics & Genomics"
S4 – Symposium on "Genomics & Genomic Analyses"
S5 – Symposium on "Mitochondrial Genetics"
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P3 – Posters on "Cancer Genetics & Genomics"
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KS-2:

NEW-GEN SEQUENCING TECHNOLOGY AND ITS IMPACT ON GENOMIC MEDICINE

Huanming Yang

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Recent breakthrough in sequencing technology, with its significantly higher throughput and lower cost, will be revolutionizing the whole field of life sciences. The next-gen sequencing technology will lead to re-designing and improvement of the on-going internationally collaborated cancer genome project and ENCODE project, as well as to the initiation of the “International 1000 Genomes Project” and projects on epigenomics and metagenomics. All these projects will have their profound impact on genetic and genomic medicine by providing more genetic markers, more reliable data for genetic variants related to common diseases, and more druggable targets. Meanwhile, rapid decoding the genomic information will bring new and more serious challenges in technology, especially bioinformatics to deal with huge amount of data, and bioethical issues, for example, how to properly protect donors’ dignity and privacy, how to avoid a new round of competition for genetic resources and gene-patenting, and how to take effective measures against genetic determination and discrimination.

KS-3:

MEDICINE – CLINICAL GENOMICS: THE PHYSICIANS' DILEMMA

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Gerald Weissman, editor of **The FASEB Journal**, quoted Claude Bernard in his editorial of April 2008 – it states: "I tell those whose path leads them toward theory or toward pure science, never to lose sight of the medical problem, which is to preserve health, and cure disease. I tell those whose career, on the contrary, guides them toward practice, never to forget that if theory is meant to enlighten practice, practice in turn should be of use to science…" The last quarter of the 20th century and the first years of the 21st have seen an explosion of genomic technology, its application to the study of human diseases and the development of potential diagnostic and therapeutic methodologies that directly impact the society we live in. The consequences of these discoveries impact the social and economic structure of humankind; these advances and the dilemmas they pose for the physician mandate attention to the message of Claude Bernard – "theory is meant to enlighten practice, practice in turn should be of use to science…" The speaker's presentation will highlight aspects of this genomic revolution, and identify clinical vignettes to exemplify issues the clinician will confront in the 21st century.

KS-4:

PUBLIC HEALTH GENOMICS PROGRAMS AT THE CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC)

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Integrating the ongoing discoveries in genomics into applied public heath programs represents a major multidisciplinary challenge. Ten years ago, CDC created the National Office of Public Health Genomics as a focal point within the Agency to collaborate with other countries and federal agencies, state health departments, academia, professional organizations, and the private sector in translating genomics research findings into practice, developing appropriate public health policies, evaluating new and emerging laboratory technologies, obtaining genetic information about the U.S. population, and fostering the expansion of successful genomic programs.

This presentation concentrates on two areas of CDC involvement: first an explanation of the background, vision, and mission of NOPHG, and second, a description of two specific CDC programs: 1) Beyond Gene Discovery, a public-private partnership and collaboration intended to genotype approximately one million single nucleotide polymorphisms to determine the prevalence of genetic variants in a large representative sample of the U.S population and 2) CDC's National Newborn Screening Reference Laboratory, the only laboratory in the world devoted to ensuring the accuracy of newborn bloodspot screening tests and supporting newborn screening laboratories in the United States and around the world.

CDC's plans for the next decade are to accelerate its research and development activities to improve genetic test evaluations and family history tools; to characterize the human genome profile of the U.S. population; and to improve existing tests and develop and evaluate new tests for detecting congenital disorders in newborns.

KS-5:

UNCOVERING DNA SEQUENCE VARIATION AND FINDING THEIR MEANING

Aravinda Chakravarti

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Human genetics is unique among the genetics sciences in that it involves the direct study of sequence variation in natural outbred populations rather than from experimental crosses. In other words, genetic studies of the human are largely possible 'by sequence' rather than 'by breeding.' Although this was a hindrance for close to 100 years, the rise of genomics has enabled a renewal of interest in human genetics, particularly its medical genetic applications. Contemporary sequencing technologies will soon allow the determination of the DNA sequence in large segments of the human genome (even the entire human genome) in individuals with defined phenotypes. The major challenge ('the holy grail') of these studies will be to assign molecular meaning to the discovered variants be they single nucleotide polymorphisms, simple insertions/deletions, copy number alterations or even major chromosomal aberrations. I will discuss studies of DNA sequencing in a number of human diseases to suggest the situations and methods by which meaning, in other words mutations, can be discovered.

KS-6:

THE SKELETAL DYSPLASIAS: CLINICAL-MOLECULAR CORRELATIONS

David L Rimoin

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The skeletal dysplasias are a heterogeneous group of disorders that result in disproportionate short stature and/or skeletal deformities. There are over 350 distinct skeletal dysplasias, which must be differentiated one from another for specific genetic counseling, prognosis and treatment. Over the past decade, there has been an explosive increase in the delineation of their biochemical and molecular defects, however the gene locus and/or molecular defect are still not known for many of these disorders. On the basis of the recent molecular findings, they can now be classified on a molecular-pathogenetic basis: Defects in extracellular matrix structural proteins; Defects in metabolic pathways; Defects in folding, processing, transport and degradation of macromolecules; Defects in hormones, growth factors, receptors and signal transduction; Defects in nuclear proteins; Defects in RNA processing and metabolism; and Defects in cytoskeletal proteins.

In the most recent international nomenclature, attempts have been made to consolidate the clinical and molecular classifications and nomenclature. In some instances, the clinically predicted families of disorders have been found to share a common etiology, whereas in other bone dysplasia families, clear locus heterogeneity was defined. It has also become apparent that previous clinical classifications of these disorders, based simply on age of onset, dysplasia versus dysostosis, or presence or absence of a single clinical feature are not valid, because a wide clinical spectrum can result from different mutations in the same gene. Thus a variety of approaches to their nomenclature and classification are necessary. It is clear that a multidimensional electronic cross-referenced classification incorporating clinical, radiological, morphological, biochemical, molecular and developmental criteria will be required for the skeletal dysplasias, as well as for most other groups of genetic syndromes.

LS-1:

MOLECULAR KARYOTYPING: FROM POSTNATAL TO PREIMPLANTATION GENETIC DIAGNOSIS

Prof. J. Vermeesch

Molecular cytogenetics and genome research laboratory, Center for Human Genetics Catholic University of Leuven, Leuven, Belgium

Genome wide array CGH, also called molecular karyotyping, enables the detection of submicroscopic imbalances in patients with mental retardation and developmental disorders. In our analysis of over 1200 patients, we detect imbalances in about 20% of the patients of which approximately 70% de novo. Since molecular karyotyping outperforms conventional karyotyping with respect to the detection of chromosomal imbalances, it has been recommended to implement this novel technology in the routine genetic diagnostic work-up of these patients. However, since most imbalances are rare and have not yet been reported before, the association of even de novo imbalances with the clinical phenotype remains to be established. In this talk, I will present evidence for de novo imbalances which are likely to be benign and evidence for the detection of inherited imbalances which are likely to be causal for the patient's phenotype. This "blurring" boundary between benign and pathogenic imbalances causes a paradigm shift in (cyto)genetic thinking and requires careful counseling of array CGH data towards patients. In addition to postnatal diagnosis, we extend CGH analysis towards prenatal and preimplantation genetic diagnosis. Our approach to molecular karyotype single cells and our recent findings on early human embryogenesis will be presented.

S1-1:

PRENATAL MALNUTRITION AND ADULT SCHIZOPHRENIA: FURTHER EVIDENCE FROM THE 1959-61 NATURAL FAMINE IN CHINA

Ming-Qing Xu[1], Wen-Sheng Sun[2], Ben-Xiu Liu[2], Guo-Yin Feng[3], Lan Yu[4], Lawrence Yang[5], Guang He[1], Pak Sham[8], Ezra Susser[5,6], David St. Clair[1,7], Lin He[1,4]

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Objective: Intrauterine malnutrition may increase risk of schizophrenia. The main evidence comes from the 1944-5 Dutch Hunger Winter and the 1959-61 Chinese famine where the most exposed cohorts, conceived or in early gestation at the height of both famines, had a two fold increased risk of developing schizophrenia in adult life. The authors tested the hypothesis in a second Chinese population that exposure to famine at conception or during early gestation increases risk of schizophrenia, and determined whether there was a risk difference between urban and rural areas.

Method: The risk of schizophrenia was examined in Liuzhou prefecture of Guangxi Autonomous region (AR). Rates were compared among those conceived, before, during and after the famine years in the area as a whole and then looking at urban and rural areas separately. Based on the decline in birth rates we predicted that those born in 1960 and 1961
would have been exposed to the famine during conception or early gestation. All psychiatric case records in Liuzhou psychiatric hospital for the years 1971 through 2001 were examined. Clinical and socio-demographic data on patients with schizophrenia were extracted by medical researchers blind to exposure status. Data on number of births and deaths in the famine years were also available and cumulative mortality was estimated from later demographic surveys. Evidence of famine was verified, and results were adjusted for mortality. Relative risks for schizophrenia were calculated for the region as a whole and for urban and rural areas separately.

Results: Mortality adjusted relative risk (RR) for schizophrenia in the prefecture as a whole for those exposed to the famine was 1.5 (1960) and 2.05 (1961) respectively. However the effect was exclusively from the rural areas RR=1.68 (1960) and RR= 2.25 (1961) with no increased risk among those born in Liuzhou city.

Conclusions: These data confirm our prediction. We observe a two fold increased risk of schizophrenia among those conceived or in early gestation at the height of famine with risk related to severity of famine conditions.

Declaration: This work was conducted for purely scientific research purposes.

S1-2:

ARRAY CGH FOR MENTAL RETARDATION AND AUTISM: CHALLENGES IN INTERPRETATION OF TESTING RESULTS

Yao-Shan Fan

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Genomic variations are the major cause of human developmental defects including unexplained mental retardation and autism. Microarray based comparative genomic hybridization (array CGH) has been used as a powerful tool in cytogenetic diagnosis. Genome-wide array CGH studies have detected a large number of pathogenic submicroscopic imbalances in patients with mental retardation and autism but also revealed a high frequency of copy number variations (CNVs) with a nature of benign or unknown clinical significance. This presentation will show our comparison and validation of different platforms of CGH arrays for diagnostic and investigational purposes. Example cases are used for illustration of the power and the limits of the technology as well as the challenges in interpretation of testing results.

S1-3:

OF MICE & MEN: PROGRESSIVE & STATIONARY RETINAL DYSTROPHIES

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Retinal dystrophies are a heterogeneous group of Mendelian disorders whose classifications are evolving as researchers better understand their phenotypes, genotypes and pathophysiology. Genetic counseling is an important part of taking care of the patient. Electrodiagnostic studies continue to be the mainstay of diagnosis, and autofluorescence imaging has become essential for better phenotyping and following disease progression.

Studies utilizing animal models in combination with progress in the field of molecular genetics have improved our understanding of pathways leading to retinal degenerations. As a result, it has become clear that genes are involved in many processes that are responsible for the symptomatology seen in these conditions. However, it is still a mystery how certain genetic defects can cause a myriad of retinal degenerations while others defects, often in the same genes, lead to much more benign conditions such as stationary night blindness. As future research uncovers new details about specific genetic defects and the discovery of more accurate animal models, we can hopefully develop gene therapeutic strategies that will one day prevent, treat, and even cure these blinding and debilitating diseases.

S1-4:

GENETICS OF CARDIAC MALFORMATIONS

Taosheng Huang

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Congenital heart disease is one of the most common major malformations in humans, contributing substantially to the financial and psychological burden of child healthcare. About one percent of children are born with heart defects, and every year, more children die from congenital heart disease than are diagnosed with cancer. A diagnosis of congenital heart disease is frightening for parents, particularly when it affects a fragile newborn. The heart is the first organ to be matured in a human fetus and if a particular congenital heart defect is compatible with fetal life, the child will be born with a defective heart. More than 300 genetic syndromes are associated with congenital cardiac defects. In this talk, we will review the genetics of congenital heart disease, how to carry out a diagnosis of the genetic causes and how to provide counseling for families with congenital heart disease.

S1-5:

STUDIES OF UROFACIAL (OCHOA) SYNDROME DEMONSTRATE A COMMON MECHANISM FOR FUCNTIONAL VOIDING DISORDERS

Cong-Yi Wang

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The Urofacial (Ochoa) syndrome (UFS, OMIM #236730) is a rare autosomal recessive disease. The patients have both urinary and facial abnormalities. The symptoms may start at very young ages, and many of the patients die in their teens to early 20s because of renal failure. The children show a dysfunctional facial expression characterized by a peculiar distortion of the face, grimacing as if in pain or sadness when they try to smile or laugh. The urinary abnormality shares the characteristic spectrum of symptoms and signs with bladder dysfunction syndrome, voiding dysfunction syndrome, elimination dysfunction syndrome and non-neurogenic neurogenic bladder. The children beyond of toilet training present with repeated episodes of urinary tract infection (UTI), dysuria, frequency, enuresis, constipation, bladder trabeculation, and reflux. Previously, we have performed a genome-wide scan using both homozygosity mapping and DNA pooling strategies and localized the defective gene to chromosome 10q23-q24. By haplotype analysis of 26 polymorphic microsatellite markers flanking this region, we have further narrowed the disease interval to 1.8Mb of genomic DNA between markers D10S1433 and D10S603. The UFS gene was then identified by mutation screening all candidate genes within the region. UFS encodes a very conserved 591-amino acid protein with restricted expression in the facial muscle and smooth muscle. The exact function for UFS is currently unknown. Our initial functional study suggests that UFS may regulate the signals derived from motor neuron implicated in the coordination of facial muscle and urinary smooth muscle contraction. Elucidation of the function for UFS would lead to the development of therapeutic strategies for voiding disorders that affect millions of children and adults world wide.

S1-6:

GENOTYPE PHENOTYPE CORRELATION STUDIES IN TUBEROUS SCLEROSIS COMPLEX

KS Au, Hope Northrup

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Tuberous sclerosis complex (TSC) is a human autosomal dominant disease characterized by hamartoma growth in multiple organ systems. Severe mental retardation with seizures is the leading cause of premature death in TSC. The estimated incidence is 1/6000 live births with approximately 2/3 of cases are de novo. Mutation in TSC1 or TSC2 is the major cause of TSC. TSC1 codes for hamartin and TSC2 codes for tuberin. Hamartin and tuberin form heterodimer to regulate RHEB/mTOR activity and subsequently regulating protein synthesis and cell proliferations.

A total of 368 TSC families in USA were enrolled between 1978-2005 for linkage study and mutation screening using Southern blot, MLPA, SSCP, HA, and direct sequencing methods. Standardized TSC features were recorded for genotype phenotype analyses. Sixty families have TSC1 mutations and 193 families have TSC2 mutations. All TSC1 mutations predicted to cause protein truncation, 30% TSC2 mutations are missense and 70% are protein truncation mutations. Genotype phenotype correlation meta-analyses on our study with two large independent studies concluded TSC2 families have higher risk for subependymal nodules (OR=3.4, CI 1.85-6.27), MR (OR=3.96, CI 2.51-6.24), forehead plaques (OR=3.29, CI 1.85-5.86), renal angiomyolipomas (OR=8.27, CI 4.36-15.7), and retinal phakoma (OR=6.94, CI 2.94-16.39). Individuals with contiguous deletion of TSC2 and PKD1 genes have a higher risk for early onset infantile polycystic kidney disease. Missense mutation on TSC2 is equally pathogenic as protein truncating mutation and do not predict disease severity. Male TSC patients have higher prevalence of neurologic features (OR=2.2, CI 1.4-3.4), and higher prevalence for LAM associated with TSC2 mutations almost exclusively in female patients than female patients. Better understanding of genotype phenotype correlation can lead to better disease management.

S1-7:

APPLICATION OF MOLECULAR CYTOGENETICS IN WOMEN WITH RECURRENT ABORTION AND SPONTANEOUSLY ABORTED ENRYOS IN WESTERN AREA OF THE KINGDOM OF SAUDI ARABIA

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Aim of the study: The purpose of the study is to assess the role, frequency and nature of chromosomal aberrations that contribute to the occurrence of repeated abortions in Saudi Arabia. It was to detect any cryptic chromosomal alterations and genetic mutations in spontaneous recurrent abortion (SRA) by using FISH technique. Generate the computerized database at KFMRC for the cytogenetic data in couples with SRA. Material and Methods: Study included 104 blood samples from a mixed population with a majority of Saudi couples. About 25 couples were referred for cytogenetic studies during 2006-2007. A sample database was created with patient's demographics including hospital number, clinic history and other lab. Test results. Out of total 52 individuals, 27 females with a history of recurrent abortion were examined. Only about 22 females of Saudi origin were selected for the analysis studies. Molecular cytogenetic techniques were used in the studies and standard cytogenetic and FISH procedures were applied. Results: Prevalence of chromosomal aberrations among individuals experiencing SRA and having been married with in relation is about 50%. Chromosomal abnormalities were detected in 3 women and 1 man, of which one was a related couple, suggesting chromosome aberrations were more frequent in women. Conclusion: This study should help physicians working in the region to realize the contribution of chromosomal abnormalities to cases of repeated fetal loss. It should also help in setting priorities of cytogenetic screening in individual cases.

S2-1:

CONTEXT DEPENDENT IMPACT OF THE Y440X CAMPOMELIC DYSPLASIA SOX9 MUTATION

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Mutations in the human, SOX9 gene are associated with the skeletal malformation syndrome, campomelic dysplasia (CD) and sex reversal. Heterozygous mutations in human SOX9 result in conductive and sensorineural deafness in some CD patients, implying a role for SOX9 in ear development. Complete inactivation of the Sox9 gene in mice results in failure of cartilage formation, presumably because of failure to express SOX9 target genes such as extracellular matrix genes, Col2a1, Col9a1, Col11a2 and aggrecan. Because all the SOX9 mutations are heterozygous, are distributed throughout the gene and appear to cause loss of function, the CD phenotype has been attributed to haploinsufficiency of SOX9. However SOX9 proteins containing an intact HMG box and a truncated activation domain may act dominant negatively by competition with the wild-type for binding to target genes and interfere with interaction with partner factors via the transactivation domain. To assess whether such mutations in SOX9 may act in a dominant interference mechanism we generated transgenic and conditional knock'in mice expressing a mouse equivalent of a CD mutation, a Y440X nonsense mutation causing premature termination within the trans-activation domain of SOX9 (Sox9Y440X). We compared the phenotypic impact of the Sox9Y440X mutation with a Sox9 null mutation. These studies point to an essential role for Sox9 in inner ear and intervertebral disc development and context dependent mechanisms for the Y440X nonsense mutation.

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S2-2:

GENETIC BASIS OF premature ovarian failure (POF)

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In premature ovarian failure (POF), ovaries cease to mature oocytes before 40 years of age. The condition is characterized by secondary amenorrhea, infertility, hypoestrogenism and elevated gonadotropin serum levels (FSH>40 IU/L). Premature ovarian failure (POF) is a heterogeneous disorder that is not rare, having at least 1% prevalence. Chromosomal causes have long been recognized – visible deletions of the X chromosome, 45,X/46,XX mosaicism, and autosomal rearrangements (balanced translocations). All are uncommon explanations. Toxins or iatrogenic causes (e.g., chemotherapeutic agents) are even rarer. Absence of explanations has led to the deduction that mutant autosomal or X-linked genes must be responsible for the majority of POF cases. With molecular technology it is now possible to search for Autosomal and X-linked genes perturbed in POF. There exist many attractive candidate genes, usually based on animal models (mice), and all are candidates for interrogation.

One predictable set of genes whose perturbations should cause POF are those encoding gonadotropins (FISH, LH) or their receptors (FSHR, LHR). Mutations in these genes are rare causes of POF, except for FSHR mutations in Finland. Other genes expressed during oogenesis are thus being considered. Interrogated by ourselves and others have been DNA binding proteins and transcription factors such as NOBOX , FIGLA and LHX8, RNA binding proteins such as NANOS, TGFβ family members such as GDF9, and G protein receptors such as GPR3. Many other genes are expressed in oocytes (AT2, KIT, NOGGIN, MIS, MISR, BAX, RFPL4). To date causative mutations have been identified in only a few genes (NOBOX, GDF9, LDX8, BMP15, POF1b and FMR1). Even for these genes, only 1-2% of POF cases show a perturbation. Thus, considerable heterogeneity - phenotypic as well as etiologic – exists in ovarian failure. Given the still limited number of explanations for POF, other genes must be sought and novel mechanisms of gene action (e.g., copy number variants) considered.

S2-3:

SELECTIVE ISOLATION OF HOMOZYGOUS MUTANT ES CELLS

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An arrayed library of ES cell clones each with a different homozygous mutation is a desirable resource for functional analysis of the mammalian genome; it can be widely used in phenotype-driven recessive genetic screens and for making genetic mosaics.

Bi-allelic (homozygous) mutations can be generated in ES cells but to date this has only been conducted on a gene-by-gene basis. Libraries of thousands of single allele gene-trap mutations can be readily generated, but these are not suitable for conducting genome wide recessive screens because of the rarity of homozygous mutants. The high rate of mitotic recombination in Blm-deficient ES cells has allowed us to generate mutation libraries in which homozygous mutant clones are enriched, but these cells are still present at a relatively low frequency (0.01%). Although screens of these libraries have been successful, they rely on strong dominant selection.

We have developed a general strategy to specifically recover the rare homozygous mutant clones from libraries of heterozygous mutations generated in Blm-deficient ES cells. First, a dual (invertable or deletable) gene trap selection vector is delivered by a *PiggyBac* transposon which expresses just one of the two cassettes, but can switch between them. Then after transient expression of Cre, the rare homozygous mutant clones can be recovered because they express both selection markers while the heterozygous mutants express just one reporter. Using this scheme homozygous mutant clones have been generated independent of phenotypic selection and more important, it can be conducted on a high-throughput method.

S2-4:

PRIMATE-SPECIFIC ZINC FINGER PROTEIN ZNF480 IS INVOLVED IN MYOGENESIS

Yuequn Wang, Yan Bai, Wuzhou Yuan, Yongqing Li, Yun Deng, Xiaoyan Mo, Xiongwei Fan, Rolf Bodmer*, Karen Ocorr, Xiushan Wu

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In the human genome, there exist a large number of specifically expanded zinc finger genes that are primate-specific. ZNF480 is one of these primate-specific zinc finger proteins and it has been used as a model to elucidate the function of these types of proteins. The ZNF480 gene is highly expressed in muscle tissue and we now show that this gene modulates muscle specific gene expression and E12/MyoD heterodimerization. Both the mRNA and protein expression levels of *ZNF480* are up-regulated during myogenesis in C2C12 cells. In addition, over-expression of *ZNF480* in C2C12 cells promotes myotube formation during skeletal muscle cell differentiation. ZNF480 also increases the mRNA level of muscle-specific basic helix-loop-helix (bHLH) transcription factors, muscle-specific gene MCK (muscle creatine kinase), and protein level of MHC, myogenin and p21. Over-expression of *ZNF480* in NIH3T3 cell enhances MCK4800-luciferase activity when cotransfected with E12/MyoD. These findings document a novel pro-myogenic role for the recently identified primate-specific ZNF480 protein.

S3-1:

INTEGRATED GENOMICS IN CANCER MEDICINE

Edison Liu

Genome Institute of Singapore

Classical approaches to the study of oncogenes and tumor suppressor genes commonly take one target, and draw associations between that gene and clinical outcome. We have pursued a different approach that engages genomic technologies, computational interrogation, and cell biology to arrive at pathway or mechanism associated expression markers which then are validated as clinically predictive. The fundamental difference is the comprehensiveness and the precision of the analyses afforded by new genomic technologies. Our results show that embedded in the expression footprint of tumors, are the composite of signatures of specific pathways and physiologic states. Many of these mechanism-associated signatures have prognostic and predictive power, and provide rationale for clinical combinations of targeted therapeutics. Using breast cancer as a model system, we have identified clinically relevant signatures for p53, estrogen receptor beta, estrogen receptor alpha, and grade that also are relevant for predicting clinical outcome. Moreover, there is evidence that these signatures extend across different tumor histologies. These genomic approaches allow for massively parallel discovery of biomarkers for any disease state, and rapid in silico validation. Each of which are candidates for imaging and therapeutic targets.

S3-2:

THE USE OF GENOMICS AND GENOME-WIDE ASSOCIATION STUDIES IN THE IDENTIFICATION OF NEW DISEASE GENES FOR ENDOCRINE LESIONS (ADRENAL HYPERPLASIA)

CONSTANITNE A. STRATAKIS, MD, DMSci

NICHD, NIH, USA

With the promise of state-of-the-art molecular technologies and the tools provided by the human genome project, a number of investigators are trying to identify molecular targets responsible for endocrine lesions. One path in this endeavor was the identification by positional cloning of genes that are mutated in rare conditions. The subject of this presentation is an update on the second path that was followed by us and others: that of using genome-wide (GW) expression analysis and GW Association (GWA) studies to find new genes. Transcriptomic analysis is a rapidly evolving technology; we can only summarize some data and use the example of our work on the adrenal gland to point out how these new technologies can be used in the identification of important genes and molecular pathways that have been identified in both normal and diseased tissue.

S3-3:

MICROSATELLITE INSTABILITY IN HEREDITARY AND SPORADIC COLORECTAL CANCER – GENETIC AND EPIGENETIC MECHANISMS

Suet-Yi LEUNG

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Inactivation of the DNA mismatch repair (MMR) system would lead to an increased mutation rate and predispose to the development of colorectal cancer (CRC) with the microsatellite instability (MSI) phenotype. The two DNA MMR genes most commonly affected are the *MSH2* and *MLH1* genes, and biallelic inactivation of either gene is needed for CRC development. These can be achieved through a combination of germline/somatic mutations in patients with the Hereditary Non-Polyposis Colorectal Cancer (HNPCC) Syndromes, or bi-allelic inactivation by aberrant promoter methylation in 15% of sporadic CRCs. Screening for MSI in CRCs constitutes a useful initial test to suggest HNPCC and the need for follow up genetic diagnosis. The distinction between germline versus somatic causes of MSI CRCs has profound implication for patient management and prophylactic screening of their family. We have identified a unique germline *MSH2* c.1452-1455delAATG founder mutation, which accounts for a significant proportion of early-onset and familial CRCs in Hong Kong Chinese, and may contribute to the observed high incidence of early-onset CRC locally. Recently, we have discovered a novel mechanism for HNPCC, due to a stably inherited germline methylation of the *MSH2* gene promoter, but with highly mosaic tissue-specific distribution of the methylated alleles (*Nat Genet* 2006;38:1178-83). Along with recent reports by other groups of germline methylation of *MLH1* in some putative HNPCC patients, epigenetic silencing is increasingly recognized to cause not only sporadic, but also hereditary cancers in humans. Germline methylation of *MLH1* and *MSH2* exhibit interesting differences in terms of the propensity for transmission to offspring and the degree of mosaicism of the methylated alleles, which have revealed for the first time the diversity and complexity of epigenetic changes as a cause of hereditary disease in humans. Recognition of these new mechanisms for genetic disease is important as the disease manifestation may deviate from Mendelian inheritance and the mosaic distribution of the methylated alleles may create problem in genetic diagnosis. Specifically, presence of methylated MLH1 promoter in CRCs are easily misinterpreted as somatic event whereas detection of methylation in corresponding normal tissue may easily be dismissed as age-related non-specific changes.

S3-4:

GENOMIC HETEROGENEITY IN MULTIPLE MYELOMA – ON THE WAY TO MAKE IT SIMPLIFIED FOR BETTERMENT OF PATIENT CARE

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Plasma cell myeloma is one of the most prevalent hematologic neoplasms worldwide. It is characterized by heterogeneity in its clinical presentation, genomic abnormalities, response to chemotherapy, and prognosis.

Studies on genomic correlation with diagnosis and prognosis in the past decade have opened a new page for myeloma patient care. Numerous genomic aberrations that are of diagnostic or prognostic in myeloma have been identified. The adverse effects of particular genomic alterations, such as TP53 deletion and some rearrangements involving the immunoglobulin heavy chain locus, including t(4;14), and 1p21 deletion are well-established. The prognostic significance of others, such as t(11;14) and deletion 13q in hyperdiploid cases, is controversial. Other commonly observed cytogenetic changes in plasma cell myeloma that have been used as genetic markers for diagnosis include gain of copies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. Recent reports suggest that amplification of 1q21 increases as disease progresses from monoclonal gammopathy of undetermined significance (MGUS) to relapsed myeloma. Thus, 1q21 amplification appears to serve as a marker of prognosis and disease progression in myeloma. However, due to the highly heterogeneous nature of the genomic abnormalities in myeloma, a standard classification system that correlates specific genomic abnormalities to prognosis has not yet well-established.

The laboratory MCIM system, which combines bone marrow morphology evaluation, cytogenetics study, immunophenotyping, and molecular analysis, has been recognized as a useful integrated tool for diagnosis and prognosis in hematological malignancies. Immunophenotypic analysis of plasma cells by flow cytometry is now performed routinely on bone marrow specimens being evaluated for plasma cell myeloma. Important phenotypic markers that can be used for diagnosis, prognosis, and management of the disease include CD10, CD20, CD38, CD45, CD44v9, CD45, CD56 and CD138. However, the patterns of expression of these markers and their association with specific genomic changes have not well been established. Compared to immunophenotypic analysis, bone marrow evaluation still remains important in myeloma diagnosis and prognosis. Markers obtained from bone marrow for response to treatment and clinical consequences in plasma cell myeloma are mainly plasma cell count, and plasmablastic morphology features. In addition, higher B2-microglobin is one of the most important chemistry prognostic factors in the malignancy. Similar to immunophenotyping, establishment of correlation of these markers with genomic abnormalities would further improve the accurate diagnosis and prognosis in myeloma and thus the cancer patient care.

It is difficult to obtain abnormal cytogenetic results in myeloma using conventional culture techniques and Giemsa banding because the neoplastic cells grow poorly in culture, even when stimulated with mitogens, such as lipopolysaccharide (LPS). Thus, the results of conventional cytogenetic studies usually show a normal diploid karyotypes, which is the karyotypes of the admixed non-neoplastic cells. In addition,

conventional cytogenetic analysis is time-consuming and labor-intensive. Fluorescence in situ hybridization (FISH) has become a powerful technique to assess a variety of hematological malignancies for known genetic abnormalities. Compared to array CGH which is recently under development for cancer patient care, FISH analysis may be the most appropriate approach, at least for time being, that would supplant conventional cytogenetic study for the routine clinical evaluation of plasma cell myeloma because of its relative ease and the availability of probes. However, given its genetic heterogeneity, it is appropriate to develop an optimal laboratory diagnostic and prognostic FISH panel for the evaluation of clinical samples.

In this presentation, results of myeloma genomic studies from commercial and academic setting each will be presented, and laboratory approach for betterment of myeloma patient care is proposed.

S3-5:

CDNA ANALYSIS DEMONSTRATES THAT THE *BRCA2* INTRONIC VARIANT IVS4-12DEL5 IS A DELETERIOUS MUTATION

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Mutation screening of the breast and ovarian cancer predisposition genes *BRCA1* and *BRCA2* is becoming an increasingly important part of clinical practice. Whereas many disease-causing mutations are associated with increased risk of cancer, the contribution of the sequence variants to cancer risk remains largely undefined. Classification of rare non-truncating sequence variants in the *BRCA1* and *BRCA2* genes is problematic, because it is not known whether these subtle changes alter function sufficiently to predispose cells to cancer development. As a result, carriers of variant with uncertain significance (VUS) and their family members cannot take advantage of the risk assessment, prevention, and therapeutic measures that are available to carriers of known deleterious mutations. The importance of accurate pre-mRNA splicing is illustrated by the fact that about 15-50% of human genetic diseases arise from mutations that affect normal mRNA splicing. Here we reported the cDNA analysis of a *BRCA2* variant IVS4-12del5. We demonstrated that the *BRCA2*IVS4-12del5* splice site variant results in the deletion of exon 5, and the gene putatively produces a truncated *BRCA2* protein of 164 amino acids instead of 3418 with the incorporation of 22 out of frame amino acids. These results support the conclusion that *BRCA2* IVS4-12del5 is a deleterious mutation. This study will shed light on the reclassification of intronic variants that do not disrupt the 5' and 3' splice sites (the GU-AG rule).

S3-6:

GERMLINE HMSH2 PROMOTER MUTATION IN A CHINESE HNPCC KINDRED: EVIDENCE FOR DUAL ROLE OF LOH

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Hereditary non-polyposis colorectal cancer (HNPCC) is a dominantly inherited cancer predisposition syndrome that is caused by germline mutations in mismatch repair genes. By screening the core promoters of hMSH2, hMLH1, and hMSH6 in 37 Chinese suspected HNPCC families, a novel germline mutation c.278_279delGT was found in the hMSH2 promoter. Its pathogenic effects were supported by the following findings: (a) it co-segregated with HNPCC-related cancers and was not present in the 220 control subjects, (b) tumors harboring the mutation lacked the expression of hMSH2 and showed high microsatellite instability, (c) it significantly decreased the promoter activity, and (d) it abolished the binding ability of the transcription factor E1A-F. Loss of heterozygosity (LOH) was found in three of the tumors studied. Intriguingly, in the tumors from patients II:1 and III:1, LOH occurred in the wild-type allele and agreed well with the traditional 'two-hit' model. In contrast, in the tumor from patient III:3, LOH occurred in the mutant allele. A pathogenic somatic mutation (c.221011G.A) was also found in this tumor; therefore, we proposed that the 'second hit' was inactivated by somatic mutation, and the mutant allele was lost during tumor progression; this provided evidence for the new hypothesis for the dual role of LOH.

S3-7:

MOLECULAR GENETICS RESEARCH OF NASOPHARYNGEAL CARCINOMA

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Loss of heterogeneity on short arms of chromosome 3 and 9 has been reported to be associated with NPC, and 3p25-26, 7q31-32, 9p21-22 were confirmed to be the smallest common LOH/deletion area. The inherited susceptibility area was shortened from 13.6cM to 12.4cM on chromosome 3p21.31-3p21.2 in 18 NPC pedigrees from the Hunan province in southern China. A lactotransferrin (LTF) gene, mapping to 3p21 was identified as a good candidate tumor suppressor gene of NPC. Using a series of large scale screening techniques from genomics, transcriptomics, proteomics and tissue microarray, we had constructed a molecular markers system in different stages of NPC: a) SPLUNC1, EBER-1, p16, p27, RASSF1A and CDH13, a group of molecular targets for the early diagnosis of NPC. b) The class forecast model consisting of up-regulated RB1, STMN1, DSP and down-regulating SERPINB6, AGTRL1, SYTL2, the biomarkers to identify between normal nasopharyngeal epithelium and NPC. c) NGX6, Ezrin, LTF, OPN, THY1 and Tiam-1, a group of candidate biomarkers forecasting the invasion and metastasis of NPC. d) Cyclin D1, Survivin and HPA, a group of biomarkers related to the prognosis of NPC. e) Bcl-2, EGFR and Ki67 proteins, a group of perfect candidate biomarkers for forecasting radiation sensitivity of NPC. f) SAA and cox-2, the candidate biomarkers monitoring NPC recurrence. g) The changes of six SNP from BRD7, NGX6, NOR1 and UBAP1, a group of the inheritance susceptibility risk factors. h) Constructing a biomarkers system of different clinical stages of NPC composed of 139 genes. In addition, we verified that nanobacteria (NB) co-exists with EB virus, and SPLUNC1 can kill it by combining with nanobacteria in NPC.These findings provide us a new mechanism of NPC carcinogenesis. **Grant sponsor:** The National Key Project of Scientific Research Program (2006CB910502), The National High Technology Research and Development Program of China(2007AA02Z170), The 111 project (111-2-12).

S4-1:

CHARACTERIZATION OF GENE COPY NUMBER CHANGES IN LOW AND HIGH GRADE GLIOMAS BY HIGH RESOLUTION ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

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Introduction: Gliomas consist of a spectrum of neoplasms that typically present as low grade brain tumors and recur as high grade malignancies. Genomic gains and losses for this evolution have been characterized at chromosomal and subchromosomal levels using low resolution techniques, typically scanning the genome with arrays containing several thousand probes. We performed high resolution array CGH to identify gains and losses in gene copy number in paired low grade and high grade gliomas to identify critical genes involved in this evolution.

Methods: Following receipt of Human Assurance Committee approval, the histopathology of biopsy tissues from craniotomies for brain tumors was reviewed. Cases with evolution from low grade astrocytomas to high grade gliomas (gliosarcomas and glioblastomas) were selected. Regions of blocks from tissues fixed in formalin and embedded in paraffin (FFPE) that contained representative tissues were identified and sectioned. DNA was extracted from FFPE sections using the TrimGen Wax Free kit and analyzed for DNA copy number changes using Affymetrix SNP250K chips. A total of 20 samples from 12 patients with gliomas were analyzed. Initial diagnostic samples paired with recurrences were analyzed in 4 patients. In three patients, low and high grade regions within the same tumor sample were compared. Two other patients were glioblastomas with prolonged survival (over 24 months).

Results: DNA from archival FFPE tissues yielded adequate coverage, as assessed by analysis of SNPs. Whereas lower grade gliomas had few regions of deletion or amplification, evolution to a high grade astrocytoma was associated the accumulation of genetic deletions, as well as amplifications. Array CGH identified genes known to be deleted or amplified in gliomas, as well as novel genes that had not been previously reported in this malignancy. Gliomas associated with a prolonged survival time contained different types of DNA copy number changes, in contrast with aggressive cases. In addition, high-grade gliomas accumulated greater number of alterations with recurrence associated progression.

Conclusions: High resolution DNA copy number profiling of the tumor genome in gliomas is a powerful resource in determining mechanisms of tumor progression over time that can be performed on archival FFPE tissues. While a common fingerprint of genomic deletions and amplifications in gliomas was not observed, the evolution of gliomas was associated with copy number changes in families of genes involved in regulation of key mechanisms that program tumor cell biology. This unbiased, genome-wide profiling carries the potential to identify genomic targets for possible therapeutic interventions in individual patients.

S4-2:

ASSOCIATION STUDIES OF COMPLEX DISEASE USING DENSE SNP SETS

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Association studies of complex disease have been revolutionized by the completion of the International HapMap Project and the availability of whole-genome genotyping technologies. We have performed regional association screens using high-density SNP sets, as well as whole-genome association analysis, on complex disorders in the Hong Kong population. For regional screens, we have developed novel methods for tag SNP selection that utilize both positional and functional information in determining the optimal choice of SNPs. For whole-genome screens, we have developed an analysis toolset that incorporates both standard association tests and implements novel approaches such as genetic sharing between subsets of individuals with similar phenotype as a way to detect rare variants. A local screen for schizophrenia susceptibility loci on chromosome 3p has revealed SNPs in a putative candidate gene with nominally significant association with schizophrenia. Joint analysis of this gene with other putative schizophrenia susceptibility genes using Multiple Dimension Reduction (MDR) has revealed significant interactions. A whole-genome analysis of Hirschsprung Disease has identified a functionally relevant gene with 2 SNPs that reached genome-wide significance level.

S4-3:

THE PARADOX OF DNA METHYLATION IN GERM CELL TUMOR

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DNA methylation is a heritable feature in eukaryotes, its exact role in mammalian development has been controversial. In our recent genome-wide analysis (GWA) of global DNA methylation on human germ cell tumors, we observed the majority of the methylated regions (78%) were in the regions without Refseq annotations, where only less than 9% of methylation is observed in the promoter regions. Subsequent comparison between normal and tumor datasets indicated only 3-4% of differentially methylated regions (DMRs) mapped to the promoter regions of known genes, such as HOX associated DMRs. We have identified a total of 197 candidate genes with promoter associated DMRs that are highly related to tumor development. Follow-up validation on the promoter associated DMRs showed no solid correlation between DNA methylation and gene expression. Among 54 DMRs located at the gene promoter region verified, only 35% demonstrated same trend between DNA methylation and gene expression, which is comparable to 37% reported. Again, the HOX gene cluster presented an interesting example in this case. Even though HOXC10 and HOXA7 were among the 39 DMRs with DNA hypermethylation, the expression trend was totally opposite. HOXC10 demonstrated decreased in expression by more than 24 fold in the tumor sample, whereas HOXA7 showed more than 46 fold increase in the same sample. The wide occurrence of DMRs in the non-coding regions is worth noting and its possible effects on chromatin structure are still unknown. The unexpected paradigm of DNA methylation in transcription has yet to be fully explored.

S4-4:

RECOMBINATION IN THE SCHIZOPHRENIA-ASSOCIATED AND POSITIVELY SELECTED *GABRB2*: IMPLICATION TO THE ORIGIN OF PSYCHOSIS

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Why does schizophrenia, clearly burdened with reproductive disadvantages, persist in humans? Hypotheses proposed to address this central paradox of schizophrenia are mainly based on phenotypic 'trade-off', maladaptation to present-day environment, or a multi-gene version of the mutation-selection balance operative in single gene disorders. Here we show, in the schizophrenia-associated gene *GABRB2*, evidence for the co-existence of two opposing evolutionary genetic factors – hot-spot recombination and directional selection. The hypothesis is advanced that a balance between these two factors, with recombination increasing and directional selection decreasing genetic diversities, gives rise to a dynamic equilibrium that maintains a steady ~ 1% incidence of schizophrenia, thereby providing a plausible explanation of the central paradox. Its identification of 'hot-spot' recombination as the major source of genetic variation instead of spontaneous mutations brings into accord the substantial uniqueness of human hot-spot patterns and schizophrenia being a specifically human disease. Given the much higher recombination rates relative to mutation rates, and the known gender differences in recombination, the hypothesis explains the higher incidence of schizophrenia compared to most single gene disorders, as well as the otherwise puzzling schizophrenia features of gender difference in disease onset and same-gender concordance. Unlike the mutation-selection balance hypothesis, the present *recombination-selection balance hypothesis* accommodates the actions of both purifying and positive selections at the same schizophrenia-associated genomic regions. Since positive selection has been reported for a number of schizophrenia genes, the present hypothesis may also be applicable to some other schizophrenia genes besides *GABRB2*.

S4-5:

GENOMIC REGIONS SUBJECTED TO STRONG SELECTIVE SWEEP AND A DATABASE OF POSITIVE SELECTION ACROSS HUMAN AUTOSOMES

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Positive selection is major driving force that shaped modern human. With advances in high-throughput methods to catalog genetic variations, population genomics has been applied to identify genomic evidence of selection. However, relatively little has been described for the degree of selective sweeps across the genome and the systematic comparisons among various statistics.

Therefore we performed a genome-wide scan for selection signals in HapMap phase II dataset. Briefly we conducted simulations to demonstrate that selective sweeps were expected to affect HE*T* average at regions with advantageous alleles, and then applied boxplot to identify low HET outlier windows based on empirical distributions of genotypes. From 2,081 clusters containing contiguous low HET windows, we detected 1,461 regions among HapMap populations as the candidates subjecting to strong selection (P_{boot} <0.01). These regions were supported by population genetic tests that outliers with high F_{ST} or strong linkage disequilibrium occurred more frequently in candidate regions than the genomic background (χ^2 test, P <0.01). Based on our results which provide new clues and methods to understand recent selective sweeps and population differentiation, we further built SNP@Evolution as an integrative and hierarchy database focusing on selection. SNP@Evolution combines F_{ST} and HET with available iHS (integrated Haplotype Score) at levels of SNPs, genes, and chromosome regions. To capture multiple signals of positive selection across the genome, empirical P values of HET, F_{ST}, and iHS of most annotated genes were computed and integrated to demonstrate outliers as shown in data query interface. The low diversity regions were detected by sliding a 100kb window in a 10kb step. The graphic user interface was constructed with Generic Model Organism Database toolkit to develop Gbrowser to illustrate various datasets. Available at http://bighapmap.big.ac.cn, we hope SNP@Evolution to become a valuable resource for studying various patterns of positive selection and population differentiation.

S4-6:

ENDINGS IN THE MIDDLE: STUDY OF AN INTERSTITIAL TELOMERE SEQUENCE (ITS) AT 22Q11.2

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By using double-strand primed in situ labeling (PRINS) technique with telomere sequences as primers, we discovered a specific telomere signal on human chromosome 22q11.2 region. Having scanned in the genome database, we found a telomere-like sequence containing a 101 tandem repetitive 9-base unit, TTAGGGAGG and TTATGGAGG, locates at 22q11.2 for a total 909 bp in length. We designed an ITS-specific primer pair based on its sequence found from genome database to perform PRINS. It resulted in a high signal frequency and specificity at 22q11.2. Furthermore, using PCR with primers flanking the ITS, we discovered different allele patterns of unrelated normal individuals and of genetic relationship in family members. Our findings indicate the presence of polymorphism for the ITS and highly suggest that the ITS could be an ideal marker for genetic studies in the future.
Whereas the relationship between the sequence organization for telomeres and their function is relatively clear, the presence of the ITS inside chromosome is far from being understood. Given that the susceptibility of chromosome 22q11 region to rearrangements is unexpectedly high as recognized on the basis of multiple congenital anomaly disorders and some malignant neoplastic diseases, for instance, del(22)(q11.2) in velo-cardio-facial/DiGeorge syndrome (VCFS/DGS), inv dup(22)(pter-q11.2) in cat-eye syndrome, t(9;22)(q34;q11.2) in chronic myeloid leukemia (CML) and t(8;22)(q24;q11) in Burkitt's lymphoma, further study may also illuminate relations between ITS and the genomic instability and chromosome rearrangements.

S4-7:

IDENTIFACATION OF A NOVEL MODIFIER OF SOX10 IN MELANOCYTE DEVELOPMENT BY A SENSITIZED GENOME-WIDE ENU MUTAGENESIS SCREEN

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Many human neurocristopathies include genetic defects of neural crest (NC) stem cells during development. In recent years a complex network of genes has been associated with pigmentation abnormalities and deafness resulting from NC stem cell-derived melanocyte deficiency. For example, Waardenburg syndrome (WS) can result from mutations either in *Sox10* or *Mitf*. We previously showed that melanocyte development critically depends on both *Sox10* and *Mitf*. We also found that $Sox10^{Dom}$/+; $Mitf^{Mi}$/+ double heterozygotes show increased dorsal and ventral white spotting compared to $Sox10^{Dom}$/+ or $Mitf^{Mi}$/+ single gene mutant. This demonstrates that a mutation in a single locus can increase WS traits in *Sox10*/+ mice. Based on the observation, we have established a sensitized genome-wide ENU mutagenesis screen in the mouse for identifying novel *Sox10* modifiers. Our screen is designed to identify ENU induced mutation(s) in additional novel genes that act synergistically with *Sox10* to increase the melanocyte defects of $Sox10^{LacZ}$/+ mice. One of phenotypes identified in this screen, named *betelgeuse*, produces a head white spotting and acts to increase the ventral white spotting in $Sox10^{LacZ}$/+ mice. Linkage analysis confirmed its location on chromosomes 3 within a 3.6 Mb region distinct from any previously identified WS loci. This study demonstrates the feasibility of sensitized screens to identify disease modifier loci.

S5-1:
MODIFIER FACTORS MODULATE THE PHENOTYPIC EXPRESSION OF DEAFNESS-ASSOCIATED MITOCHONDRIAL DNA MUTATIONS

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Mutations in the mitochondrial DNA are one of the most important causes of hearing loss. Of those, the homoplasmic A1555G and C1494T mutations at a highly conserved decoding region of in the 12S rRNA have been associated with aminoglycoside-induced and non-syndromic hearing loss in many families worldwide. The A1555G or C1494T mutation is expected to form novel 1494C-G1555 or 1494U-A1555 base-pair at the highly conserved A-site of 12S rRNA. These transitions make the secondary structure of this RNA more closely resemble the corresponding region of bacterial 16S rRNA. Thus, the new U–A or G-C pair in 12S rRNA created by the C1494T or A1555G transition facilitates the binding of aminoglycosides, thereby accounting for the fact that the exposure to aminoglycosides can induce or worsen hearing loss in individuals carrying these mutations. In the absence of aminoglycosides, a wide range of variable penetrance and expressivity of hearing loss was observed in matrilineal relatives among and within these families carrying these mtDNA mutations. These suggested that modifier factors including nuclear modifier genes, mtDNA variants/haplogroups (background sequence) contribute to the phenotypic variability. Recently, we identified the first nuclear modifier gene TRMU, involved in mitochondrial tRNA modification, for the phenotypic manifestation of the A1555G mutation in the Arab-Israeli/European (Spanish/Italian) pedigrees. Most strikingly, we showed that the TRMU A10S mutation suppresses the aminoglycoside toxicity associated with the A1555G mutation. Furthermore, audiological, genetic evaluations and sequence analysis of complete mitochondrial genome of 53 Chinese pedigrees carrying the A1555G mutation indicated that variants $tRNA^{Cys}$ T5802C, $tRNA^{Ser(UCN)}$ G7444A, $tRNA^{Arg}$ T10454C, $tRNA^{Ser(AGY)}$ C12224T, $tRNA^{Glu}$ A14693G, $tRNA^{Thr}$ T15908C, G15927A and ND5 T12338C influence the phenotypic expression of the A1555G mutation. In addition, we showed that the Eastern Asian haplogroups B5B and F2 increase the risk for deafness associated with the A1555G mutation. These data could potentially aid the genetic counseling of families, and are important for prevention and treatment of this disorder. In particular, the ototoxicity is likely preventable through a combination of evaluating family history and molecular analysis of 12S rRNA gene in susceptible individuals. Every individual, prior to an administration of drugs, should be examined for a family history. If member(s) of a family suffered from aminoglycoside-induced deafness, others should be screened for those 12S rRNA mutations. Those, who are positive for those 12S rRNA mutations, should be warned that they are at risk for aminoglycoside ototoxicity and avoided for the use of those drugs. Thus, we can predict which individuals are at risk for ototoxicity, improve the safety of aminoglycoside antibiotic therapy, and eventually decrease the incidence of deafness.

S5-2:

PEDIATRIC LIVER FAILURE CAUSED BY MITOCHONDRIAL DNA DEPLETION

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Mutations in at least nine nuclear genes are currently known to cause mtDNA depletion. A recent report suggests that mitochondrial DNA depletion is the most common cause of mitochondrial hepatoencephalopathy (Sarzi, et al., 2007; Dimmock et al Hum Mut 2007). The typical presentation is severe neonatal onset liver failure. Among them, deoxyguanosine kinase *(DGUOK)* mutations are the most common cause of mitochondrial hepatoencephalopathy. Patients with severe *MPV17* mutations present predominantly infantile liver failure, and death occurs before neurological symptoms become apparent (Wong et al. Hepatology 2007). The majority of patients with mutations in DNA polymerase γ (*POLG*) have predominantly CNS problems followed by liver failure in early childhood (Alpers syndrome). Patients with mild mutations may have relatively mild disease, but liver failure can be precipitated by drug treatment or viral infection. Thus, defects in these genes predispose the individual to liver failure.

Clinical features may overlap among patients with molecular defects in different genes. On the other hand, heterogeneous clinical phenotypes have been observed in patients with mutations in the same gene. *POLG* mutations have been identified in severe Alpers syndrome as well as less severe autosomal recessive and dominant forms of PEO, the sensory ataxic neuropathy with dysarthria and ophthalmoparesis (SANDO), and of spinocerebellar ataxia with epilepsy (SCAE). Thus, sequence analysis of the gene and the identification of the pathogenic mutations provide a definitive diagnosis of the disease. Up to date, about 7, 40, and 100 mutations have been reported in *MPV17, DGUOK*, and *POLG* gene respectively. Defects in these genes cause mtDNA depletion that can be detected in the affected tissue, such as liver. Recently, a MitoMet oligonucleotide array has been developed for the simultaneous detection of copy number changes in both mtDNA and nuclear genes responsible for mtDNA depletion.

S5-3:

MOLECULAR MECHANISM UNDERLYING DIFFERENTIAL APOPTOSIS BETWEEN HUMAN MELANOMA CELL LINES UACC903 AND UACC903(+6) REVEALED BY MITOCHONDRIA-FOCUSED CDNA MICROARRAYS

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Human cutaneous malignant melanoma (CMM) currently reigns as the most deadly form of skin cancer, responsible for 75% of all skin cancer-related deaths. CMM frequently results from the transformation of melanocytes in the skin and its incidence worldwide has doubled in the past 20 years. High resistance to treatment is a unique hallmark of CMM, although the mechanisms by which CMM cells protect themselves against induced apoptosis remains largely unknown. Our investigation focuses on the molecular mechanism underlying sensitivity and resistance of CMM cells to induced apoptosis.

Using the CMM cell line UACC903 and chromosome 6-mediated suppressed cell line UACC903(+6), we revealed a significant difference in apoptosis G2/M arrest between these two cell lines before UV irradiation. In response to UV treatment, UACC903 cells displayed a significant increase in G2/M arrest and linearly increase in apoptosis, in contrast to the exponential increase in apoptosis of UACC903(+6). Applying our recently developed human mitochondria-focused cDNA microarray, we identified 154 differentially-expressed genes of which 104 displayed differential expression in response to UV irradiation in both cell lines. qRT-PCR validated expression of 93.3% of microarray datapoints tested (10 genes at 3 time-points, n=30). Proapoptotic (n=16) and antiapoptotic (n=3) genes showed significant ($p<0.05$) difference in expression and were mapped to known survival-apoptosis signaling pathways. Without UV treatment, 10 proapoptotic genes (BAK1 [6p21.3], BCAP31, BNIP1, CASP3, CASP6, FAS, FDX1, FDXR, TNFSF10 and VDAC1) and 3 antiapoptotic genes (BCL2L1, CLN3 and MCL1) were expressed at higher levels in UACC903(+6) than in UACC903. At 12-hours after the UV treatment, 8 of these 13 genes were down-regulated in UACC903(+6) but all were upregulated in UACC903. Western analysis confirmed the expression changes of BAK1, BCL2L1, FAS and MCL1 proteins. The FAS ligand (FASLG)-induced cell death was dose- and time-responsive, demonstrating apoptotic sensitivity and resistance of UACC903(+6) and UACC903, respectively. The siRNA knockdown of BAK1 protein in UACC903(+6) significantly ($p=0.01$) increased survival by 113.5±6.44% and abolished the FASLG-induced cell death ($p=0.23$). Thus, our study revealed the molecular survival-apoptosis signaling pathways underlying the differential apoptosis between UACC903 and UACC903(+6) cell lines and have implications for therapeutic research.

S5-4:

SLC26A4 C.919-2A>G VARIES AMONG CHINESE ETHNIC GROUPS AS A CAUSE OF HEARING LOSS

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Mutations in the SLC26A4 gene are second only to GJB2 mutations as a currently identifiable genetic cause of sensorineural hearing loss (SNHL). In most areas of China, genetic testing for SNHL is unavailable due to limited knowledge of the mutation spectrum. While SLC26A4 c.919-2A>G (IVS7-2A>G) is a common mutation among some Asian populations, the mutation prevalence among various ethnic groups within China has not been studied. DNA specimens from 3,271 subjects with SNHL from 27 regions of China were genotyped for the c.919-2A>G mutation by PCR/RFLP. Normal hearing controls from Han (n=185) and Uigur (n=152) populations were also tested. Overall, 408 subjects with SNHL (12.5%) carried at least one c.919-2A>G allele, with 158 (4.8%) homozygotes and 250 (7.6%) heterozygotes. Within the subpopulations examined, the rate varies from 0% to 12.2% for c.919-2A>G homozygotes and from 0% to 17.6% for heterozygotes. Based on this cohort, Chinese subjects with SNHL appear to have a relatively higher c.919-2A>G frequency than that of other Asian populations. These results demonstrate that a simple and efficient genetic test for the c.919-2A>G mutation alone would identify the molecular cause in up to 12.2% of individuals with SNHL in certain regions of China. Those who are negative for the c.919-2A>G mutation would be candidates for further mutational analysis of SLC26A4 or other deafness-related genes. This would greatly improve genetic diagnosis and counseling for at least 2 million Chinese individuals and family members with SNHL in China, and many more ethnic Chinese in other countries.

S5-5:

CLINICAL AND GENETIC FEATURES OF LEBER'S HEREDITARY OPTIC NEUROPATHY IN CHINESE POPULATION

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited disorder leading to the rapid, painless and bilateral loss of central vision. Despite the remarkable progress in the identification of mtDNA mutations associated with LHON in European and North American populations, less has been known in the Chinese population. First, we have performed a clinical and genetic characterization of 535 Chinese pedigrees with LHON. Second, we have initiated a systematic and extended mutational screening of mtDNA in this clinical population, especially in ND4 G11778A, ND6 T14484C and ND1G3460A mutations. We showed that 182 pedigrees carrying the G11778A mutation, 6 families carrying the G3460A mutation and 28 pedigrees had T14484C mutation. Indeed, the frequency of these primary LHON mutations is lower than those of other clinical populations. Other mtDNA mutations are associated with other Chinese pedigrees. These families exhibited a wide range of clinical phenotypes. The age-at-onset ranged from 7 to 40 years old, with the average of 17 years old. Approximately 30% of male and 12% of female matrilineal relative in these families suffered from vision loss. These matrilineal relative in these pedigrees also exhibited a wide range of severity of vision loss, varying from blindness to normal vision. These suggested that nuclear and mitochondrial modifiers or environmental factors may play a role in the phenotypic manifestation of LHON associated with these primary mtDNA mutations in these Chinese families.

S5-6:

CLINICAL ANALYSIS OF 8 MELAS PATIENTS WITH MTDNA A3243G POINT MUTATION

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Introduction: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is the most common mitochondrial disease due to mitochondrial DNA (mtDNA), A3243G tRNA (Leu(UUR)) gene mutation for the majority of cases. The clinical phenotype is very variable characterized by the degree of heteroplasmy and multi-systemic involvement of mutant load. **Objective:** Eight molecular confirmed MELAS patients of age ranged from 2 to 54 years were recruited and studied clinically. **Result:** The clinical spectrum is very broad from most frequent features of myopathy(88%) and lactic acidosis(88%) to those less common namely deafness (50%), GI disturbance (38%), failure to thrive (25%), diabetes mellitus (25%), left ventricular hypertrophy (25%) and renal failure (13%). **Conclusion:** Since positive family history are substantiated by molecular testing of maternal blood in at least 50% of our series, thorough clinical evaluation and molecular testing of mtDNA with various tissue samples is crucial to provide accurate genetic counseling for family screening of maternal relatives.

S5-7:

INCIDENCE OF MITOCHONDRIAL D-LOOP ALTERATIONS IN TOBACCO ASSOCIATED ORAL SQUAMOUS CELL CARCINOMA

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Mitochondrial DNA (mtDNA) is known to be more vulnerable to mutations as compared to the nuclear DNA due to a lack of histone protection, weak repair capacity and constant oxidative stress. Displacement -loop (D-loop) region is considered as the mutational 'hotspot' and is widely studied in different types of cancers. We wished to examine the frequency of D-loop polymorphisms and mutations in tobacco associated oral squamous cell carcinoma in patients from eastern part of India. The D-loop hypervariable region I (HVI) and hypervariable region II (HVII) were studied in 20 oral cancer patients and in oral scrapings of 20 healthy individuals. HVI and HVII regions of D-loop were amplified, sequenced and analyzed for polymorphisms and the mutations. Sequencing of the tumor DNA revealed 10/20 (50% samples) showed mutations in the D-loop. Of these 40% of the alterations were heteroplasmy in the C-tract of the HVII region. 2/20 (10%) showed missense mutation in the HVI region and 2/20 (10%) samples showed missense mutations in the HVII region. One sample showed missense mutation in both HVI as well as HVII region. Novel variations were also observed in both tumor tissues and healthy individuals. High incidence of D-loop mutation was observed in oral squamous cell carcinoma.

S6-1:

NANOPARTICLE-BASED GENE THERAPY FOR METABOLIC DISORDERS: HEPATIC DELIVERY OF MINI-CIRCULAR DNA FOR COMPLETE CORRECTION OF PHENYLKETONURIA

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We report that organically modified silica (ORMOSIL) nanoparticles complexed with plasmid DNA (nanoplex) are capable of efficient hepatic gene transfer in mice after intravenous injection under normodynamic pressure, which led to peak levels of transient transgene expression that were comparable to those achieved by hydrodynamic injection of naked plasmid DNA. We further show that comparable levels of sustained transgene expression in vivo could be achieved when mini-circular DNA vectors were used, being injected either hydrodynamically as naked DNA or normodynamically as a nanoplex. Hepatic transfer of the nanoplexes was achieved without serum proinflammatory cytokine response as well as other systemic and organ toxicities. ORMOSIL nanoplexes containing mini-circular DNA expressing murine phenylalanine hydroxylase were then administered intravenously under normodynamic pressure in a mouse model of Phenylketonuria (PKU). Complete and persistent correction of the hyperphenylalaninemic and hypopigmentation phenotypes was achieved after a single administration of the nanoplexes in all treated mice. The results suggest that this nanoparticles-based gene delivery technology can be developed in the future for effective and safe treatment of patients with PKU and other genetic disorders that are secondary to hepatic deficiencies.

S6-2:

A TUMOR-TARGETING NANOIMMUNOLIPOSOME FOR SYSTEMIC CANCER GENE THERAPY

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A platform nanodelivery system has been developed comprising a self assembled, biodegradable, cationic liposomal nanoparticle, which bears targeting molecules that home to the surface of tumor cells. When systematically administered, this nanocomplex can efficiently and selectively deliver nucleic acid-based molecular therapeutics, diagnostic MRI contrast agents, and small molecules to not only primary tumors, but also metastases in animal models of a number of human cancers. The nanodelivery of imaging agents results in a marked improvement in the sensitivity and resolution in detecting minute metastatic lesions. The tumor targeting delivery of various molecular therapeutics has also been shown to dramatically synergize with conventional radio- and chemotherapies. This approach is now entering clinical trials. There is ongoing collaborative effort to correlate integrated high-resolution imaging and physical characteristics with the biological activity of these nanoparticles to accelerate in vivo optimization of the nanodelivery system.

S6-3:

ENZYME REPLACEMENT THERAPY FOR LYSOSOMAL STORAGE DISEASES

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Enzyme replacement therapy (ERT) in Japan was approved for Gaucher disease in 1996. Subsequently ERTs for Fabry disease, mucopolysaccharidosis (MPS) type 1, Pompe disease and MPS type 2 were approved in Japan. In Gaucher disease ERT will lead to improvement of hepatosplenomegaly and hematological abnormality. ERT for Fabry disease will inhibit the progression of renal dysfunction and improve cardiomyopathy. ERT for MPS type 1 is effective in improving joint stiffness, chronic heart failure and respiratory dysfunction. ERT for Pompe disease will improve the life span and motor function for the infantile type. ERT for MPS type 2 will improve the range of movement in some joints.

As described above the patients with lysosomal storage diseases (LSD) have received benefit from ERT. In this presentation I will show the clinical improvement in various patients with LSDs.

S6-4:

RESTORATION OF MITOCHONDRIAL FUNCTION

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Mitochondrial defects have been reported to associate with more and more human diseases. However, there is no effective treatment for such kind of condition. In this study, different approaches are utilized in restoring the mitochondrial function in cells with mitochondrial deficiency. We found that change(s) in the expression of a certain nucleus-encoded factor(s) can compensate for the absence of the certain subunit of respiratory components, indicating alternative pathway of respiratory complex assembly. We further showed that the yeast NDI1 enzyme can improve the mitochondrial capacity in cells with a defective respiratory system and protect the cells from oxidative stress and cell death. Our work suggested novel therapeutic approaches for mitochondrial diseases.

S7-1:

ADULT STEM CELLS AND NANOMATERIALS IN SKELETAL TISSUE ENGINEERING AND REGENERATION

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Nanoscale materials are the fundamental building blocks and functional subunits of cells, including subcellular organelles and extracellular matrix components. Currently, there is growing recognition of the importance of understanding and incorporating nanobiology into biomedical applications. This issue is of particular importance in the emerging field of regenerative medicine, the goal of which is to develop methods to repair, replace, and regenerate diseased, injured, or non-functional tissues. Towards this goal, stem or progenitor cells have been considered a highly desirable candidate cell type, because of their expandability and potential to be induced toward specific cell differentiation lineages. A key requirement in tissue engineering and regenerative medicine is that ultimately the “regenerate tissue” needs to be a three-dimensional structure. In weight-bearing musculoskeletal tissues, this requirement is particularly critical. Musculoskeletal disorders affect one out of seven Americans. This severe disease burden underscores the need to develop novel and effective treatment protocols. This lecture will present the promises as well as the challenges in the field of skeletal tissue engineering and regeneration, specifically the application of adult stem cells and nanomaterial scaffolds. The biology of human adult mesenchymal stem cells, particularly the mechanisms regulating their proliferation versus differentiation into specific lineages, is intricately regulated by cell-cell interactions, signaling by extracellular bioactive factors, and transcriptional and epigenetic activities. More importantly, the extracellular matrix milieu provides critical cues, both architectural and structure-dependent, to guide cell-based tissue morphogenesis. We have developed biomimetic and biodegradable nanofibrous biomaterials to serve as scaffolds for cell-based tissue engineering. Information on the fabrication and biological basis of the scale-dependent bioactivities of the nanofibrous scaffold will be presented. Cell-nanofibrous constructs are currently being developed for the engineering of cartilaginous tissues, including articular cartilage and intervertebral disc. In conclusion, tissue engineering represents a unique, emerging inter-disciplinary research field that is a natural platform for life scientists, engineers, and clinicians working together to advance regenerative medicine.

S7-2:

HEDGEHOG SIGNALING IN EPIDERMAL STEM CELL DEVELOPMENT AND TUMORIGENESIS

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Hedgehog (Hh) signaling has been implicated in the differentiation, proliferation, and/or survival of stem cells in both invertebrates and vertebrates. In human, deregulation of the Hh pathway leads to various developmental disorders and cancer. While Hh signal transduction is relatively well understood in Drosophila, the molecular mechanism underlying the mammalian pathway is still poorly defined. Using the mouse as a model system, we have been studying the function of Gli transcription factors, which are nuclear transducers of Hh signaling. In mammalian cells, control of the degradation of Gli2 activator and the proteolysis of Gli3 into its repressor form define Hh signaling response. In this report, I will present our analysis of two regulators of Gli transcription factors, Suppressor of fused (Su(fu)) and Kif7. By biochemical and genetic analyses, we demonstrated that both Su(fu) and Kif7 regulate the formation of Gli3 repressor. In addition, Kif7 also plays a role in the degradation of Gli2 activator and Kif7 null mice display elevated Hh response due to stabilization of Gli2. Unexpectedly, we uncovered a novel function of Su(fu) in the stabilization of Gli transcription factors, demonstrating that it also acts as a positive regulator essential for maximal Hh signaling response. We showed that overactivation of the Hh pathway in the epidermis results in hyperproliferation of stem and progenitor cells, which ultimately leads to skin tumorigenesis. However, inactivation of Su(fu) or Kif7 alone in the epidermis does not result in skin tumorigenesis. These observations illustrate the importance of Hh signaling in the control of the stem cell compartment in the epidermis and reveal distinct roles for Su(fu) and Kif7 in the regulation of Hh signaling response in epidermal stem and progenitor cells.

S7-3:

MESENCHYMAL STEM CELLS FOR SKELETAL TISSUE ENGINEERING

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It was not until the late 80's that tissue engineering was regarded as an independent branch of science. The term tissue engineering was initially defined by the attendees of the first National Science Foundation of the United States sponsored meeting in 1988 as "application of the principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationship in normal and pathologic mammalian tissues and the development of biological substitutes for the repair and regeneration of tissue or organ function". In 1993, Langer and Vacanti summarized the early development in this field and defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissues or organ function. The exercise of interdigitating these different functional talents into a coherent device has produced the working definition of tissue engineering: "Tissue engineering is an art and science by which synthetic compounds are manipulated into anatomically and/or functionally specific architectures and, when required, may be integrated with biologically active agents and/or living cells such that resultant properties of the whole are precisely suited to support the specific cell life prescribed for recipient tissues". Consequently, tissue engineering has now emerged as a potential alternative to tissue or organ transplantation. Based on the above mentioned principles of tissue regeneration, reconstructing segmental bone defects after resection of malignant bone tumors, a long-standing challenge for orthopaedic surgeons was an excellent demonstration of the application of mesenchymal stem cells (MSCs) in orthopaedic tissue engineering. With the increased knowledge of MSCs, we have demonstrated that it is possible to reconstruct segmental bone defects using a tissue engineering approach. Also, the combination of nano-technology with MSCs for skeletal regeneration is another good example. We have cultured MSCs on biomimetic electrospun Type 1 collagen nanofiber scaffold, making the composite an excellent advanced therapy product for reconstruction of flat bones. Future efforts will be made to further validate the safety and efficacy of applying MSC technology for skeletal regeneration in both pre-clinical and clinical settings.

S7-4:

DIRECTED DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO CARDIAC LINEAGE

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** Equal contributions*

Embryonic stem cells (ESCs) are derived from the inner cell mass of blastocyst. They can self-renew and are pluripotent, meaning that they have the ability to proliferate indefinitely and to differentiate into all cell types in our body, including cardiomyocytes. Therefore, ESCs can potentially act as a source of cardiomyocytes for therapy. However, in order to put ESC-derived cardiomyocytes into clinical uses, there are a number of hurdles which need to be overcome. One of the biggest obstacles for practical usage of these ESC-derived cardiomyocytes is the current inability to obtain cardiomyocytes in sufficient quantity. In this presentation, some of the studies conducted in our laboratory which aim to direct ESC differentiation into cardiac lineage using biochemical and genetic approaches will be discussed. These studies can provide insights into the possible ways of increasing the yield of cardiomyocytes from ESCs.

S7-5:

PROBING THE MOLECULAR MECHANISMS OF CELL FATE RESTRICTION

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A gene's transcriptional output is the combined product of two inputs: diffusible factors in the cellular milieu acting in trans, and chromatin state acting in cis. Here, we describe a strategy for dissecting the relative contribution of cis versus trans mechanisms to gene regulation. Referred to as trans-complementation, it entails fusing two disparate cell types and searching for genes differentially expressed between the two genomes of fused cells. Any differential expression can be causally attributed to cis mechanisms because the two genomes of fused cells share a single homogenized milieu in trans. This assay uncovered a state of transcriptional competency that we termed 'occluded', whereby affected genes are silenced by cis-acting mechanisms in a manner that blocks them from responding to the trans-acting milieu of the cell. We identified occluded genes in a variety of cell types. Importantly, occluded genes in a given cell type tend to include master triggers of alternative cell fates. Furthermore, the occluded state is maintained during cell division and is extraordinarily stable under a wide range of physiological conditions. Chromatin analysis shows that DNA methylation contributes causally to the occluded state and also suggests the involvement of HP1. These results support the model that the occlusion of lineage-inappropriate genes is a key mechanism of cell fate restriction. The systematic description of occluded genes by our assay offers a novel molecular definition of cell type, and provides a hitherto unavailable functional readout of chromatin state across the genome.

S7-6:

X-CHROMOSOME VARIATIONS IN A PARTHENOGENETIC HUMAN EMBRYONIC STEM CELL LINE

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HLA homozygous parthenogenetic human embryonic stem (phES) cells are important materials in the study of gene function and epigenetic mechanisms. They may also be important sources in cell therapy. However, many functions and characteristics of phES cells are still unknown. In the present study, some imprinted genes (H19, IGF2, SNRPN and GNAS), XIST gene, DNA methylation, DNA fingerprinting, HLA typing and X chromosome inactivation status were analyzed in a phES cell line that has been established in our laboratory. Human embryonic stem (hES) cell lines derived from fertilized eggs were used as controls. We found that there were no significant differences in the characteristics and functions between phES cell line and hES cell lines except X chromosome inactivation status. Normal hES cells with XX karyotype had active X chromosome and inactive X chromosome irrespective of its passages. However, phES cells had two active X chromosomes before 20 passages. After 20 passages, some cells lost one active X chromosome. The proportions of cells with only one active X chromosome gradually decreased until 35 passages in which all cells had only one active X chromosome, showing a karyotype of 45X0. These results indicate that genetic and epigenetic statuses are not stable in the phES cells, which may affect the application of these cells to the treatment of human diseases.

S7-7:

HUMAN FEEDER CELLS FOR PROLONGED CULTURE OF HUMAN EMBRYONIC STEM CELLS

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Human fibroblasts from different tissues had been used as feeder cells for human embryonic stem cells (hESCs), so that hESCs cultured in an exno-free system could be closer for clinical standards. Here, we derived 8 fibroblast lines from fetal skin, and after treated with mitomycinC these cells were prepared as feeder layers for culturing hESCs. Results showed that all of the 8 lines had good ability for supporting growth of undifferentiated hESCs. One line, named as hEF-dly-2, had been used as feeder cells for over 3 years, on which 4 hESC lines (chHES-8, 20, 22, 32) had been continually cultured for no less than 80 passages. All of the 4 hESC lines showed normal karyotypes, low differentiating proportion (<15% by AKP staining), and could be induced to all three germ layers. RT-PCR showed that hEF-dly-2 expressed high level of FGF2, Follistatin, activinA, BMP2, FGF7, and low level of BMP2, BMP4, as well as 9 kinds of Wnts genes at different expression levels. These genes were involved in pathways which were widely associated with cell proliferation, cell cycle and apoptosis, cell adherence and differentiation. They might partly explain why feeder cells could support hESCs, and gave clues for improving feeder-free culture system. Moreover, fibroblasts from fetal skin were better than fibroblasts from lung or foreskin during our hESCs culture. We concluded that fetal skin derived human fibroblasts could well support hESC growth, and the gene expression profile of hEF-dly-2 might serve as a good referrence for selecting suitable feeder cells.

S8-1:

NONINVASIVE PRENATAL DIAGNOSIS OF TRISOMY 21 USING CELL-FREE FETAL NUCLEIC ACIDS IN MATERNAL PLASMA

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Our laboratory reported the presence of circulating cell-free fetal DNA in maternal plasma in 1997. Since then, we have been exploring the biology and diagnostic applications of this noninvasive source of fetal genetic material. We are especially interested in exploring the noninvasive prenatal detection of fetal chromosomal aneuploidies such as trisomy 21. One approach that we have recently developed concerns the use of placental mRNA in maternal plasma. We identified that one of such transcripts is from the PLAC4 gene on chromosome 21. Through the measurement of allelic ratio of a single nucleotide polymorphism (SNP) on PLAC4, we have shown that fetal trisomy 21 could be detected in maternal plasma with a sensitivity of 90% and a specificity of 96.5%. These figures suggest that this method might be the most accurate single marker-based approach for the noninvasive detection of fetal trisomy 21 yet developed. The main limitation of this strategy is that it is only applicable to fetuses that are heterozygous for the SNP. Through the development of additional SNP markers on PLAC4 and other placental-specific gene on chromosome 21, one could further expand the population coverage of this approach. We have also recently investigated the use of digital PCR-based techniques for further improving the performance of this noninvasive strategy. We are optimistic that this and other plasma nucleic acid-based approaches would increasingly impact on the clinical practice of prenatal diagnosis in the near future.

S8-2:

RECOVERING INTACT FETAL CELLS FROM MATERNAL BLOOD

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Definitive noninvasive diagnosis of fetal disorders can be achieved by analysis of intact fetal cells in maternal blood. Substantial progress has been made with this approach, and clinical introduction appears near.

I. Intact Fetal Cells

Intact fetal cells in maternal blood (1 fetal per 10^7 or 10^8 maternal cells) are rare. Thus, one must enrich the sample for these rare fetal cells. The process first requires specific collection and transport techniques, as well as targeting for a specific fetal cell type. Fetal trophoblasts, lymphocytes, granulocytes, or nucleated red blood cells (NRBCs) are candidates, but the latter is currently being pursued. Analysis of the fetal chromosomal complement in interphase cells relies on fluorescent in situ hybridization (FISH) using chromosome-specific probes.

In work now performed at Biocept, Inc., we are approaching this problem using MEMS devices (MicroElectroMechanical System). MEMS devices are based on capturing cells by attachment chemistry and fluid dynamics. Attachment is facilitated through antibodies linked to the surface of a hollowed device (75µ x 12mm x 30mm). Within the device vertical posts are randomly placed to create a flow path that will perturb fluid (and cells) passing through. Cell density and gravity further direct cells independent of the fluid flow, additionally maximizing potential to contact capture antibody. Antibody to glycophorin-A is used to enrich for embryonic or fetal cells. FISH (21-specific; X- and Y-centromeric probes) is then performed within the device (FirstCEE^{TM}), using standard fluorescent microscopy.

In pregnancies with a male fetus, fetal cell detection occurs in 90 – 95% of pregnancies as validated by a Y interphase signal. Presence of a male fetus can be confirmed independently by PCR for a Y sequence. Detecting autosomal trisomy (21) in these pregnancies is reliable. XY cells (false positive) have almost never been scored in pregnancies carrying a female fetus.

In order to confirm fetal cell origin in pregnancies with a female fetus, fluorescent staining based on presence of epsilon hemoglobin is used to distinguish fetal XX from maternal (non-epsilon) XX cells. A remaining problem is preserving integrity of chromosome-specific FISH probes in the presence of epsilon staining.

S8-3:

PREIMPLATATION GENETIC DIAGNOSIS (SCREENING) FOR CHROMOSOME ABNORMALITIES

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Preimplatation Genetic Diagnosis (PGD) or Screening (PGS) are established techniques used in conjunction with in-vitro fertilization to identify the embryos with higher risk of having genetic defects. The process involves removing one blastomere from a day 3 embryo (6-8 cell stage), performing the testing on this single cell, and transferring the embryos least likely of having the tested genetic defects on day 5. PGD has been used to detect single gene disorders by PCR or screen for chromosome abnormalities (including aneuploidy and unbalanced chromosome rearrangements) by fluorescence in situ hybridizations (FISH). With proper biopsy and fixation techniques and high standard quality control for FISH and PCR, PGD/PGS has been shown to increase pregnancy rate, reduced spontaneous abortion rate, and reduce the incidence of birth defects in patients with certain indications. Working with multiple infertility centers across the United States, we have developed strategies using FISH for aneuploidy screening and unbalanced chromosome rearrangements detection since 2002. The current technical challenges, proper quality control standards and potential future technical advances will be discussed.

S8-4:

NEWBORN SCREENING FOR LYSOSOMAL STORAGE DISORDERS

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The lysosomal storage disorders (LSDs) are a group of over 40 rare genetic disorders due to defects in lysosomal hydrolase enzymes. The pathogenesis is related to the accumulation of substrate in specific tissues, resulting in progressive degenerative disease. Some have central nervous system involvement. Clinical symptom onset is variable, ranging from early infancy to adulthood. The age of onset and rate of progression is largely related to the residual enzyme activity. Most are inherited in an autosomal recessive fashion. Newborn screening (NBS) for inborn errors of metabolism has been one of the most successful public health initiatives introduced in the last 50 years, starting with phenylketonuria in the 1960s. The basic principles of newborn screening are that the infants are phenotypically normal at birth, that presymptomatic therapeutic intervention is available and will result in decreased morbidity and/ or mortality and that there is a high throughput test available, preferably utilizing the dried blood spot filter paper sample used universally worldwide for blood-based testing.

Treatment for several of the LSDs with enzyme replacement therapy is now available, making them amenable to newborn screening. Several blood-based methods have been developed: single fluorescent enzyme assays, multiplexed tandem mass spectrometry (MS/MS) using novel substrates and internal standards, currently for 4 LSDs and multiplexed immune quantification for 11 LSDs. Testing for Krabbe disease was introduced in New York state (USA) in 2007 (MS/MS). A pilot program for Pompe disease and Fabry disease is underway in Taiwan and several other programs are planned for the near future utilizing MS/MS testing for 4 LSDs (Washington and Illinois states in the US and in Austria).

Further expansion of NBS is expected to follow. In order to fully evaluate the effect of NBS for these disorders on long term clinical outcome and the optimal timing of therapeutic intervention, protocols need to be developed for ongoing assessment after an initial abnormal screening result. Because of the rarity of the LDSs, international collaboration is essential.

S8-5:

PREIMPLANATION GENETIC DIAGNOSIS FOR 20 CARRIERS WITH ROBERTSONIAN TRANSLOCATION

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Preimplantation genetic diagnosis (PGD) is able to preclude the embryo from chromosomal abnormalities or gene defects before implantation. Here we describe our experience in PGD of Robertsonian translocation. Twenty infertile couples with Robertsonian translocation were performed 23 PGD cycles. After conventional introcytoplasmic sperm injection (ICSI), blastomere biopsy was carried out in cleavage-stage (day 3) embryos by laser zona drilling. The blastomeres were treated by hypotonic solution NaAC/BSA, followed by fixed solution 0.05% Tween-20/0.01N HCl and fresh methanol/glacial acetic acid. Fluorescence in situ hybridization (FISH) was performed by relevant locus-specific probes (including chromosome 13, 14, 15, 21 and 22). Embryos were further cultured to day 5 and normal blastocysts were transferred. A total of 365 oocytes were retrieved, and 297 (85.3%) out of 348 matured oocytes were fertilized by intracytoplasmic sperm injection (ICSI). Single blastomere biopsy and FISH analysis were successfully carried out in 96.3% (308/320) and 96.4% (297/308), respectively. Among 133 normal or balanced embryos, 45 embryos were transferred in 23 cycles. Seven clinical pregnancies were established, no spontaneous miscarriages happened. 8 healthy babies were delivered in 6 single and 1 twin pregnancies. Our results showed that PGD might be an ideal assisted reproductive technique for Robertsonian translocation carriers with infertility.

S8-6:

MOLECULAR GENETIC DIAGNOSIS AND PRENATAL DIAGNOSIS OF TUBEROUS SCLEROSIS PATIENTS IN CHINESE

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Tuberous sclerosis complex (TSC) is a dominantly inherited disease of high penetrance, characterized pathologically by the presence of hamartomata in multiple organ systems. Well known clinical manifestations include epilepsy, learning difficulties, behavioral problems, and skin lesions. Many patients have renal lesions, usually angiomyolipomata, which can cause clinical problems secondary to hemorrhage or by compression and replacement of healthy renal tissue, which rarely causes end-stage renal failure. Cysts, polycystic renal disease, and renal carcinoma can also occur.

To prevent TSC suffered-baby born, we played molecular genetic diagnosis for the proband and other patients in 7 pedigrees that come to our hospital during from 2004 to March, 2008 using PCR-DHPLC-sequencing. Then we played prenatal diagnosis in two pedigrees and two health babies had born. We identified 8 mutations in 8 patients of 7 pedigrees. Seven of the mutations, S1420T (TSC2, missense), CAT^^{383}TCGtGAT (TSC1, small insert), AG^^{374}ACCTTgGA (TSC2, small deletion), M322T (TSC1, missense), D450E (TSC1, missense), Intron 27 +1 ds g>a (TSC2, splicing mutation), CCA^^{652}GAGagAGG (TSC2, small deletion) were novel mutations that didn't report in HGMD (table not shown). The other one mutation was known one in HGMD.

S9-1:

PROSPECTS AND PROGRESS IN DEVELOPMENT OF THERAPIES FOR LYSOSOMAL STORAGE DISEASES

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During the past decade, the lysosomal storage diseases have been the focus of intensive efforts to develop enzyme, gene, substrate reduction, and molecular chaperone therapies. For Gaucher, Fabry, Pompe, MPS 1, MPS II, and MPS VI, enzyme therapies are currently approved and available, but very expensive. Substrate reduction therapies are approved and in development for several diseases as are molecular chaperone approaches. This presentation will focus on new developments in the bioproduction of treatment agents, and studies in mouse models of Gaucher disease, a prototype systems for the development and evaluation of new therapies. The results to be discussed will indicate the potential for use of a variety of approaches for the treatment of visceral and CNS disease in the human conditions.

S9-2:

DISORDERS OF CARNITINE AND FATTY ACID OXIDATION PATHWAY IN CHINESE

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Metabolic defects in the fatty acid oxidation pathway (FAOD) represent an important group of IMD. Furthermore, early diagnosis and appropriate treatment reduce morbidity and mortality. Over 10 years' experience in the metabolic clinic provided an unique opportunity to examine the spectrum of FAOD in Chinese.

Our research led to the discovery of the gene, *OCTN2* (MIM#603377), causing defect in carnitine uptake in primary carnitine deficiency (卡尼丁缺乏症, CDSP) {Tang, 1999 #1}. Subsequent investigation into samples collected from Taiwan revealed a common founder mutation of OCTN2 among Southern Chinese {Tang, 2002 #2}. The results indicate that CDSP is a common IMD in Southern Chinese with an incidence of up to 1/40,000.

Carnitine-acylcarnitine translocase deficiency (卡尼丁穿透障礙, MIM#212138) is a severe disease and usually lethal. This defect was first discovered in an American-Chinese patient. Subsequently, an identical splicing mutation (IVS2-10T/G) was found in another British-Chinese patient {Hsu, 2001 #5}. Now this mutation is recognized as a common mutation in Chinese and ante-natal diagnosis had been performed for affected families seen in this clinic.

Another common FAOD in Chinese is multiple acyl-CoA dehydrogenase deficiency (戊二酸血症二型). It can be caused by mutations in at least 3 different genes (*ETFA, ETFB, and ETFDH*), all involve in electron transfer for the fatty acid oxidation reactions. Mutations in *ETFDH* were found in the majority of cases.

Short-chain acyl-CoA dehydrogenase deficiency (短鏈脂肪酸去氫酵素缺乏症) and carnitine palmitoyltransferase II deficiency (卡尼丁結合酵素缺乏症第二型) are interesting disorders with incomplete penetrance and variable phenotypes. Clinical presentations range from completely asymptomatic, adult muscular phenotypes to infantile neuromuscular disease. Both defects have been attributed to genetic polymorphisms that are prevalent in the general population. Carriers of these polymorphisms predispose to neuromuscular disease or complications after viral infection. These examples may represent the boundary of single gene Mendelian genetics and polygenic complex trait.

This laboratory also developed new functional assays to analyze the flux rate of fatty acid oxidation in cultured fibroblast to replace the existing isotopic assay. Hopefully, it will become one of the preferred methods for making definitive diagnosis for this group of diseases.

S9-3:

MEDICAL APPLICATION OF aCGH: CLINICAL EXPERIENCE OF 14,000 CASES

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Array comparative genomic hybridization (aCGH) is a novel technique enabling high-resolution screening of submicroscopic chromosomal imbalances. It is becoming an essential and a routine clinical diagnostic tool and is gradually replacing conventional cytogenetic methods. We evaluated > 14,000 patients using targeted chromosomal microarray analysis (V5, V6 BAC and V6 BAC emulated OLIGO array) ((http://www.bcm.edu/geneticlabs/cma/). This year, we expanded the array from a 44K to a 105K OLIGO array in V7. aCGH is designed to detect aneuploidies, somatic mosaicism, deletions, duplications, and subtelomeric or other unbalanced chromosomal rearrangements. Overall detection rates for clinical relevant genomic imbalances range from 8-21% in individuals with normal results from standard chromosome analysis. A markedly improved detection rate was noted in a subset of neonates (28 days or younger) with overall detection rate of 18% and as high as 21% when the V6 OLIGO array was used. Although aCGH uncovers numerous copy number variations of unclear significance scattered throughout the human genome, aCGH holds the promise of being the first line diagnostic tool in identifying chromosomal as well as submicroscopic imbalances in patients with global developmental delay, mental retardation, autism, multiple congenital anomalies, and dysmorphism.

S9-4:

CLINICAL APPLICATION OF ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION TECHNOLOGIES: THE EMERGING NEW ERA OF CLINICAL CYTOGENETICS

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The emergence of microarray-based comparative genomic hybridization (aCGH) is revolutionizing the current practice of clinical cytogenetics. aCGH is a recently developed technology that evolved from standard CGH on metaphase spreads. There are at least three different platforms for aCGH, namely BAC/PAC- based arrays, SNP-based arrays, and oligo-based arrays, each of which carries certain advantages and disadvantages. Two different array designs have been used clinically, targeted arrays that emphasize specific genomic regions and genome-wide arrays that cover the entire genome relatively evenly. This talk will summarize our experiences of using aCGH for diagnoses of multiple congenital anomalies (MCA), developmental delay (DD), and mental retardation (MR), for phenotype-genotype correlations, and for the diagnosis/prognosis of cancer, and address the pros and cons of different array platforms. With aCGH, we demonstrate a detection rate of 17.4% for confirmed pathogenic copy number variations (CNVs) and 24.6% for total CNVs in patients with MCA, DD, and MR using Agilent 4x44K arrays. Using targeted arrays, we were able to define the deletion breakpoints of a group of Williams syndrome patient, and correlate the phenotype differences with different but overlapping deletions. We have also started to apply custom designed arrays that combined targeted and genome-wide arrays in the diagnosis/prognosis of patients with cancer. We have identified chromosomal aberrations that were missed or misdiagnosed by both conventional cytogenetics and FISH in cancer patients. Our experiences as well as others demonstrate that aCGH is far superior to conventional cytogenetics and FISH in the diagnosis and phenotype/genotype correlations of genetic disorders and cancer. aCGH is not only revolutionizing modern cytogenetics but also empowering physicians with ever expanding diagnostic capabilities. With increasing array resolution and decreasing array cost, combined with its potential for automation, aCGH will soon become the primary and preferred method for the diagnosis of copy number variations.

S9-5:

MOLECULAR TESTING OF SUDDEN CARDIAC DEATH IN YOUNG PEOPLE AND CHILDREN

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Heart disease is the most common cause of an unexpected sudden death in all age groups. The mechanism is generally a ventricular tachyarrhythmia. The underlying pathology is usually coronary heart disease in people aged 30 or over, but can also be one of the familial well-defined cardiomyopathies such as hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular dysplasia or long QT syndrome. The two main causes of sudden death in children and young athletes are hypertrophic cardiomyopathy (HCM) and long QT syndrome (LQTS). Loss of consciousness and family history of sudden cardiac death should persuade the physician to consider DNA testing and examine the patient's relatives. Gene-based diagnosis would provide accurate identification of those who have a heritable disorder; a diagnosis which should prompt appropriate medical management and genetic counseling.

S9-6:

HBUB1/HSMAD2 GENE IN SPONTANEOUS ABORTION EMBRYOS WITH NUMERICAL CHROMOSOMAL ABNORMALITY

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Objective: To research the mechanism of hBUB1/hMad2 gene in the developing of spontaneous abortion embryos with numerical chromosomal abnormality. **Methods:** The results of quantitative real-time RT-PCR and Western blot method were used to display the mRNA and protein level of hsMad2/hBub1 gene both in spontaneous abortion embryos with numerical chromosomal abnormality (experimental group) and with numerical chromosomal normality (control group). To construct recombinant shRNA plasmids targeting hBub1/hsMad2 gene and to inhibit the expression of endogenous hBub1/hsMad2 genes in embryonic cells; interference efficient was demonstrated by FQ-PCR and Western-blot; the rate of cell proliferation-inhibited was measured by MTT assay; cell-cycle was assessed by FCM. **Results:** Western blot analysis showed that comparing with the control group, the protein level of hBUB1/hsMAD2 in the experimental group was decreased significantly. Recombinant the expression of hBub1/hsMad2 gene in embryonic cells was significantly and specially inhibited by shRNA plasmids, and the rate of cell proliferation- inhibited was increased at 48 h after transfected. Efficient shRNA can decrease G0/G1 and S phase cells, and increase G2/M phase cells. **Conclusions:** Down-expression of hBub1/hsMad2 gene leads to the inhibition of cell proliferation and the alert of cell-cycle. It also probably plays an important role in the development of spontaneous abortion embryos with numerical chromosomal abnormality. It made a foundation to detect valuable clinical criterion.

S9-7:

SUBTELOMERIC IMBALANCES SEEN IN HONG KONG 2002-2008

Albert C.F. Lam, **Kent K.S. Lai**, **Ivan F.M. Lo**, **David K.H. Chan**, **Edgar W.L. Hau, Stephen T.S. Lam**

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It has been fifteen years since the fist description of “cryptic translocations” in patients with Mental Retardation. Subtelomeric rearrangement is now recognized to be an important cause of a previously unrecognized group of mental retardation patients with normal routine karyotyping. The condition is well published in overseas publications, but Chinese studies are scarce. Our objectives were to evaluate the prevalence of subtelomeric rearrangements in Chinese patients with idiopathic mental retardation, and to delineate the various subtelomeric deletion syndromes in Chinese patients. From 2002 to 2008, we recruited 485 Chinese patients from the Genetic Counselling Clinic who presented with idiopathic Mental Retardation and had a normal karyotype into the study. Thirty-seven patients (7.63%) were found to carry a clinically significant subtelomeric rearrangement. In seven of these families, one of their parents carried a balanced subtelomeric translocation (18.9%). In Conclusion, Subtelomeric rearrangement leading to chromosomal imbalances is a significant cause of mental retardation in Hong Kong Chinese patients, recognition of which allows adequate genetic counseling and offer of prenatal diagnosis to affected families.

S10-1:

GENETICS OF CRANIOFACIAL DISORDERS

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Cranial anomalies include deformation disorders such as plagiocephaly, disruption malformations such as the true craniosynostosis syndromes. Craniosynostosis is a defect of the skull caused by early fusion of one or more of the cranial sutures and affects 3-5 individuals per 10,000 live births. Craniosynostosis can be divided into two main groups: syndromic and nonsyndromic. Nonsyndromic craniosynostosis is typically an isolated finding that is classified according to the suture/s involved. Syndromic craniosynostosis is associated with various dysmorphisms involving the face, skeleton, nervous system and other anomalies and is usually accompanied by developmental delay. More than 180 syndromes exist that contain craniosynostosis. Secondary effects of craniosynostosis may include vision problems and increased intracranial pressure, among others. The molecular basis of many types of syndromic and non-syndromic coronal craniosynostosis is known and diagnostic testing strategies will often lead to a specific diagnosis.

Review of the development of the face permits an understanding of the underlying embryological and molecular basis of many facial disorders. These include disorders of: 1) Midface hypoplasia associated with hypotelorism (closely spaced eyes) e.g. holoprosencephaly, a disorder associated with defects of other midline structures, 2) Delayed closure defect/ incomplete migration of lateral structures towards midline associated with hypertelorism (widely separated eyes) e.g. frontonasal dysplasias, 3) Pharyngeal arch disorders associated with mandibular micrognathia which is the initiating cause for the Pierre-Robin sequence which is a component of several syndromes including Stickler, velocardiofacial and 4) lateral facial anomalies associated with hypoplasia e.g. hemifacial microsomia, Treacher Collins and Nager syndrome.

An understanding of the molecular and embryological basis of craniofacial anomalies is important for genetic counseling, prognosis, clinical management and surgical planning.

S10-2:

APPROACHING NEW GENES FOR X-LINKED MENTAL RETARDATION

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X-linked mental retardation (XLMR) is a highly heterogeneous genetic condition including over 140 syndromic and nonsyndromic disorders. More than 60 genes have been implicated in XLMR. The rapid progress over the past decade in identification of genes and mutations for XLMR has led to a better understanding of XLMR. In a previous report, we defined a novel XLMR syndrome in a family with six affected males with mental retardation, muscle atrophy, pigmentary abnormalities and ptosis, and two obligate carrier females in three generations, and mapped the defective gene to the region Xp11-Xp22 by linkage analysis using STR markers. Research on another nonsyndromic XLMR family, which included eight affected males and five obligate carrier females in three generations, revealed that the defective gene was located in Xq27.3 to Xq terminus. To identify the defective genes for the two families, oligo-Array CGH was performed. No deletion or duplication was detected in the probands of either family. To rule out a small deletion and/or duplication, X chromosome tiling array analysis was carried out. Suspected deletions were detected, however, these deletions could not be confirmed by PCR analysis. The expression profile of X-linked genes in the proband's lymphoblasts was surveyed by X chromosome cDNA array in the first XLMR family. The expression of at least 50 genes, such as FMR1 and DMD gene, was reduced significantly (>3 folds), and another 22 genes, such as UBE2A, SYP, and SYN1 gene, had significantly increased expression (>3 folds). Promoter analysis of these genes by Transcription Element Search System indicated more than 30 transcription factor genes on the X chromosome to be candidate genes. RT-PCR sequencing analysis of ten of the candidate genes did not detect any mutation. A similar approach with the second XLMR family also failed to detect any mutation. Thus, the defective genes in these two families still await identification. Interestingly, reduced expression of the FMR1 gene and increased expression of UBE2A, SYP, and SYN1 genes could be associated with CNS symptoms and mental retardation in the first XLMR family, while reduction in expression of DMD gene may be the cause of the muscle atrophy. Current knowledge of XLMR genes will be also discussed.

S10-3:

MOLECULAR GENETICS OF LIMB MALFORMATIONS

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Congenital limb malformations (CLM) occur in 1 in 500-1000 human live births, representing one of the most common birth defects in human population. They exhibit a wide spectrum of phenotypic manifestations and may present as an isolated trait or as part of a syndrome. Among various hand and foot phenotypes, polydactyly and syndactyly are most frequently observed. CLM can be caused mutations in the genes involved in limb development. Many genes responsible for CLM, including the well-known India hedgehog gene for brachydactyly type A1, have been identified in the last decade. These discoveries not only added to our understanding of the molecular mechanism of limb development but also improved the genetic counseling and molecular diagnosis of CLM.

HOXD13 and SHH play a critical role in early limb development. We found that mutations in the HOXD13 gene caused syndactyly type V and a novel brachydactyly-syndactyly syndrome. We identified genomic duplications involving the long-range, limb-specific SHH enhancer in triphalangeal thumb-polysyndactyly syndrome (TPT-PS) and syndactyly type IV. In addition, we detected pathogenic mutations in various types of CLM, including brachydactyly types B and C, combined syndactyly type I and brachydactyly type B, preaxial polydactyly, proximal symphalangism, split-hand split-foot malformation (SHFM), and synpolydactyly. I will present these works by giving an overview on the phenotypes and pathogenic mutations.

S10-4:

GENETIC DEFECT OF EDA GENE UNDERLIES NON-SYNDROMIC OLIGODONTIA

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Tooth agenesis is one of the most common developmental anomalies in man. So far success has only been made in identifying the genes involved in syndromic or rare dominant forms of tooth agenesis, while the genes and defects responsible for the majority of cases of tooth agenesis, especially the common and less severe forms, are largely unknown. Recent studies showed that mutations in EDA gene, which has been previously identified as causing a syndromic agenesis （X-linked hypohidrotic ectodermal dysplasia）, were detected in two families with X-linked non-syndromic hypodontia. Notably, all affected males in these families exhibited isolated oligodontia, while almost all of female carriers showed normal or milder phenotype. We hypothesized that EDA gene could be responsible for non-syndromic oligodontia in general sporadic male patients. We performed EDA gene screening for 16 unrelated sporadic male patients with non-syndromic oligodontia. Four novel mutations of Ala259Glu, Arg289Cys, Arg334His, and Thr338Met were identified in five out of 16 male probands, indicating that genetic defect of EDA gene underlies non-syndromic oligodontia in male patients. The patterns of tooth agenesis in these related subjects with defined EDA mutation were analyzed using comparative statistical analysis of tooth agenesis in EDA, MSX1 and PAX9. Statistically significant differences ($p<0.001$) were observed at eight positions. The resulting data of congenital absence of maxillary and mandibular central incisors, lateral incisors and canines, with consistent retention of maxillary and mandibular first molars, appears as a pattern of tooth agenesis, suggesting the presence of an EDA mutation.

S10-5:

PROMOTING THE QUALITY OF GENETIC TESTING IN CLINICAL AND PUBLIC HEALTH PRACTICES

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Genetic tests play an increasingly important role in clinical and public health practices with their expanding contribution to disease diagnosis, risk prediction, health management, treatment optimization, prevention of adverse drug response, and population screening for disease. Quality assurance of genetic testing has been one of the areas that have received great attention worldwide, both in individual countries and by international organizations. In countries and regions that have adopted the international standard ISO 15189 (Medical laboratories – Particular requirements for quality and competence) into their quality system frameworks for medical laboratories, some have included genetic testing in the realm of laboratory accreditation and others are initiating such processes. In 2007, the Organization for Economic Cooperation and Development published Guidelines for Quality Assurance in Molecular Genetic Testing, to promote minimum standards and best practices internationally for molecular genetic testing and facilitate mutual recognition of quality assurance frameworks. In the Unites States (U.S.), laboratories performing genetic testing for health care and health assessment purposes are subject to the Clinical Laboratory Improvement Amendments (CLIA) regulations. This presentation will provide an overview of regulatory and voluntary oversight systems for laboratory genetic testing, and will highlight several efforts of the Division of Laboratory Systems in the U.S. Centers for Disease Control and Prevention in this area, including 1) developing guidance on good laboratory practices for ensuring the quality of genetic testing; 2) improving the availability and quality of genetic testing for rare diseases and facilitating successful translation of tests from research settings to clinical laboratories; 3) improving the availability of appropriate and characterized reference materials for quality control, proficiency testing, test development, and research for genetic testing; and 4) improving the effectiveness and usefulness of genetic test reports, educational initiatives, and informational resources to support appropriate use of genetic testing. In addition, an update will be provided on the effort of the Hong Kong Accreditation Service to develop accreditation criteria for medical genetics laboratories in Hong Kong, China.

S10-6:

CLINICAL, NEUROPATHOLOGICAL AND GENETIC STUDIES OF CHINESE PATIENTS WITH INFANTILE NEUROAXONAL DYSTROPHY

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To delineate characteristics of Chinese patients with infantile neuroaxonal dystrophy (INAD), a rare autosomal recessive neurodegenerative disorder, with pathogenic gene identified in 2006. Clinical investigations including physical examinations, laboratory examinations, neurophysiological study and neuroimaging were performed in ten patients with clinical diagnosis of INAD, as well as neuropathological analysis for specimens of skin, sural nerve or muscle. All patients were screened for mutations in *PLA2G6* gene encoding iPLA$_2$-VI A (cytosolic Ca^{2+}-independent phospholipids A$_{2,}$ group VIA). All cases showed the typical clinical features of INAD. The presence of axonal swelling, which is a hallmark of pathological finding in INAD was found in skin biopsy specimens from three cases. Twelve different *PLA2G6* mutations were identified, including 9 novel mutations- 6 missense mutations (R39Q, V371M, G373R, K545E, R591W and A657V), one mutation abolishing the normal start codon (c.1A>G), one nonsense mutation (R70X) and one splice site mutation (IVS10+1G>A). Only three genetic studies on INAD patients have been published up to date, with totally 44 different PLA2G6 mutations identified. Nine novel mutations found in this study greatly broadened the spectrum of *PLA2G6* mutations. Therefore, genetic analysis is another useful tool for definitive diagnosis of INAD.

S10-7:

RENAL DYSPLASIA – COARCTATION OF AORTA. A NEW ASSOCIATION/SYNDROME: A REPORT OF 6 CASES

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Renal dysplasia is a congenital disorder of the kidneys, characterized by undifferentiated mesenchyme, immature collecting tubules and abnormal organization and is nearly always cystic. Aortic coarctation is defined as a narrowing of the aorta in the area where the ductus inserts. It may be seen as an isolated defect or associated with congenital abnormalities of the aortic valve and the left heart. The concurrent findings of aortic coarctation and renal dysplasia have not been described previously. We report a series of 6 cases with aortic coarctation and renal dysplasia with different degree of severity and outcomes.

Case series (table not shown): The 6 cases were of mixed ethnic background. Family history of renal problems was noted in 3 of the 6 patients and one case was the product of a consanguineous marriage (first cousins). All but 1 had bilateral multicystic renal dysplasia detected antenatally and only in 2 was the coarctation detected prenatally. One patient had facial features consistent with Potter sequence. Three others had common facial features with a high flat forehead, large fontanelles, deep-set eyes, hypertelorism, short noses, slightly low-set ears, single palmer creases and deep-set nails. There was no evidence of severe intrauterine growth retardation or other major birth defects. All had normal karyotypes (patient 3 had a familial inversion of chromosome 18 inherited from his mother) and no deletion at 22q11.2. One pregnancy was terminated, one child died at 3 days of life, one survived and has end-stage renal failure and mild-to-moderate developmental delay and the other 3 survived without major issues following repair of the coarctation.

The combination of renal dysplasia and congenital heart defects is rare and has been reported in a variety of chromosome abnormalities, single gene disorders (Meckel syndrome) and VACTERL association. However, the combination of renal dysplasia and coarctation of aorta is a rare event and can represent a hitherto new condition with yet unknown mode of inheritance. Parental consanguinity in one of our cases suggests autosomal recessive mode of inheritance.

S11-1:

PROSPECTIVE STUDY OF WARFARIN DOSAGE REQUIREMENTS

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Polymorphisms in CYP2C9 and VKORC1 have been shown to associate with warfarin dose requirements and could be used to predict warfarin dose. We conducted a prospective study in which warfarin dose was prescribed based on CYP2C9 and VKORC1 polymorphisms in 108 Han Chinese patients without prior warfarin treatments. Using the genotype-based dosing, 83% of patients reached stable, therapeutic INR within two weeks of treatment initiation and none of the patients developed clinical bleeding or thromboembolic event. 10% (11) of patients with INR>4 and no clinical bleeding were detected during this study. At 12 weeks, 69 % of the patients' maintenance doses matched the prediction. Dosing algorithms incorporating genetic factors, age and body surface area were developed which could explain up to 62% of the total variation (R2 of 0.62).This study demonstrated that pharmacogenetics-based dosing can improve the time to stable, therapeutic INR, reduce adverse events and achieve high sensitivity.

S11-2:

MOLECULAR GENETICS OF PRIMARY IMMUNODEFICIENCES (PID)

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More than 120 distinct genes have been identified whose abnormalities account for more than 150 different types of PID (J Allergy Clin Immunol 2007; 120:776). In Hong Kong, we have provided mutation analysis for 31 such genes of the more prevalent PIDs for Asia. The study of these PID patients has provided insights into (1) our immune system, including its interactions with pathogens, (2) immune tolerance, (3) inflammation and (4) cancer predisposition. Some PIDs predispose to infections with certain specific pathogens only, such as MSMD to mycobacteria and salmonella; and mutations in UNC93B1 and TLR3 to HSV encephalitis. Defects in (a) genes involved in apoptosis such as Fas, FasL, caspase 10 and 8, (b) transcription regulator for thymic tolerance AIRE and (c) T-cell transcription factor FOXP3, result in autoimmunity phenotypes of ALPS, APECED and IPEX, respectively. Mutations of CIAS1 (gene encoding cryopyrin, involved in leukocyte apoptosis and IL-1 processing) result in 3 autoinflammatory phenotypes (MWS, FCAS and CINCA). Defects in genes involved in DNA damage response, such as ATM, MRE11, NBS1 and BLM, result in phenotypes of reduced serum immunoglobulin and genome instability, with increase in malignancy.

Genotype-phenotype correlation has been demonstrated for WAS, whose gene WASP mutations can result in 3 phenotypes, i.e. classical WAS, XL thrombocytopenia and XL neutropenia. In-vivo mutation reversion has also been demonstrated.

Apart from high-penetrance genetic mutations, more prevalent but low-penetrance polymorphisms, such as that resulting in MBL deficiency has also been associated with susceptibility to infectious diseases, including SARS (JID 2005; 191:1679) and progression of HBV infection (Hepatology 2005; 42:1037) as well as predisposition to SLE and RA (A&R 1996; 39:706/1998; 41:1663/2000; 43:1679).

S11-3:

GENOMIC SEARCH FOR THE HIGH MYOPIA GENES

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Myopia is the most common eye disorder worldwide and the prevalence in Asia may exceed 65%. High myopia (HM) is defined as refractive error ≤-6.00 D. There is a high and increasing prevalence of HM in the Chinese population in Hong Kong, now affecting more than 10% adult population. Heredity is a major contributing factor of high myopia. While no myopia gene is still to be known, 15 chromosomal loci were mapped and two candidate genes were suggested. We have conducted a series of studies to map the myopia-associated locus and identify the myopia gene(s). Patients and family pedigrees with high myopia and normal control subjects were recruited after complete ophthalmolscopic investigations. They were all free from other eye diseases or systemic disorders such as diabetes. We have narrowed a myopia locus at 18p to the region flanking D18S476 and identified TGIF, which is located within the region, as a possible candidate gene. The 488T allele of a polymorphism P163L (488C>T) was found significantly less frequent in patients and therefore could confer protective effect against high myopia. For PAX6, a recently reported putative myopia gene, one sequence change in the promoter region was found to be significantly higher in high myopia patients. PAX6 may be associated with high myopia. Further, our whole genome scanning on high myopia pedigrees with fine mapping and haplotype analysis, have led to linkage in critical regions in 12q21.33 and 5p15. One is a narrowed region from 12q21.31-12q22 by haplotype analysis in the family, which overlapped with the reported myopia locus MYP3. A candidate gene, lumican, within this region was screened for sequence alternations in the myopia patients but no significant results were obtained. It is excluded as a myopia gene. The novel genetic locus on 5p15 is linked to region 5p15.33-p15.2 with a 17.45cM interval. We have also excluded linkage of known genes in this region with high myopia. Our results provide new information to aid in the identification of the high myopia gene(s).

S11-4:

GENETIC COMPLEXITY OF ANGELMAN SYNDROME IN CHINESE

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Angelman syndrome (AS) is a relatively common syndromic form of neurodevelopmental disorder. The incidence is about 1 in 10,000 live births, and it affects both males and females. The underlying genetic defects occur at an imprinted region on chromosome 15q11-13. About 70% of the patients are the result of a microdeletion of that region on the maternally derived chromosome 15. 10-15% of patients are due to paternal uniparental disomy 15 (patUPD15). About 10% are due to mutation of a gene, UBE3A, which shows tissue-specific imprinting. Finally, a small number of patients are due to imprinting defects. The great majority of cases are sporadic; it seldom recurs within the family. Familial cases are usually due to UBE3A mutations or imprinting centre defects.

Typical AS patients have severe global developmental delay, autistic features, absent speech, early seizures, ataxic gait, happy disposition and unexplained bouts of laughter (hence the other name Happy Puppet syndrome). The other clinical telltale signs that may be present are fair hair and fair complexion, microcephaly, brachycephaly and prognathism. Atypical patients are also found who usually have a milder and less recognizable phenotype.

Clinical Genetic Service (CGS) is a tertiary referral centre that provides genetic diagnosis and counselling for neurodevelopmental disorders. Genetic testing for AS has been provided since mid-90's, first with fluorescence in-situ hybridization (FISH) to look for microdeletion, followed by the introduction of methylation study to examine the methylation status at the 15q11-13 region, and microsatellite analysis to look for UPD15. Here we summarize the clinical and genetic findings of the thirty AS patients in Hong Kong.

S11-5:

IDENTIFICATION OF A LOCUS FOR X-LINKED DOMINANT NON-SYNDROMIC HEARING IMPAIRMENT (DFN2) IN A LARGE CHINESE FAMILY

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X-linked hearing impairment is clinically and genetically a heterogeneous disease accounting for less than 2% of nonsyndromic hearing impairment. To date, 6 X-linked nonsyndromic hearing loss loci have been identified and one of these genes have been cloned. We identified a five generation Chinese family characterized by non-syndromic sensorineural, postlingual and progressive hereditary hearing impairment. The affected males presented severe to profound deafness and age onset occurred in 5-14 years, rather the affected females had less severity hearing loss than males with onset in the fourth decade of life. A maximum two-point LOD score of 3.99 at theta=0 was obtained for marker *DXS8096* in this family by linkage analysis. Haplotype analysis placed the locus within a 5.4 cM genetic interval defined by marker *DXS8020* and *DXS8055*, overlapping with the DFN2 locus on chromosome Xq22. Several candidate genes in this region have been screened but none of them has yet been shown to be mutated in this family.

S11-6:

FUNCTIONAL CHARACTERIZATION OF THE REGULATORY REGION OF HUMAN CD2AP PROMOTER IN HEK 293 CELLS

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Background: The mRNA of CD2 associated protein (CD2AP) was found to be changed in congenital and acquired glomerulosclerosis. Promoter plays an important role in the regulation of gene expression, but the characterization of the human CD2AP promoter has not been systematically analyzed in HEK 293 cells. **Aims:** This study was to analyze in detail the promoter of human CD2AP in HEK 293 cells. **Methods:** The transcriptional initiation sites were identified by 5' RACE from RNA in HEK 293 cells. Promoter activities were detected by series deletion and mutational luciferase analysis. For transactivation assay, Sp1 and Sp3 expression plasmids were transfected. **Results:** Multiple transcriptional start sites (TSSs) were identified. Progressive deletion analysis from both 5' and 3' ends revealed two kinds of promoter activity. One basic promoter activity located within 500 bp upstream of ATG. Fragments of further upstream 100 bp or more increased the promoter activity by 5-fold. Two Sp1/Sp3 sites were in this region. Mutation of these two sites reduced the transcriptional activity by 50%. Overexpression of Sp1 increased the activity, whereas that of Sp3 decreased the activity. Deletion of 9 SNP sites and 3 Lmx1b sites did not change the transcriptional activity. **Conclusion:** CD2AP have multiple TSSs. Two promoters of CD2AP were identified. Sp1/Sp3 binding sites play a critical role in the CD2AP activation. These findings should facilitate studies on the mechanism regulating the expression of this gene and on the clinical mutational and polymorphism analysis.

S11-7:

TRANSCRIPTION COMPLEXITY GENERATED BY CO-TRANSCRIPTION AND INTERGENIC SPLICING IN MAMMALS

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It has been sporadically reported that transcription may read through the intergenic region and stopped at various sites of the downstream gene. These fused transcripts also be spliced and translated into one chimeric protein. However, it is not clear if this phenomenon of co-transcription and intergenic splicing is a rare situation of adapted transcription error or another mechanism of one gene for multiple products. To answer this question, we searched all available mRNAs in public databases to computationally detect the co-transcriptional mRNAs genome-wide by aligning mRNAs to refseqs to find transcripts spanning two individual genes. Over 8 million human and 2 million mouse ESTs and full-length cDNAs were aligned to their refseqs respectively. After removing artificial ESTs, 582 and 198 co-transcripts were obtained. These fused mRNAs were grouped into five major patterns according to their 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 fused transcripts. By RT-PCR and sequencing, we validated 23 (51%) and 18 (40%) fused transcripts out of 45 randomly-chosen co-transcripts from the two species. Interestingly, about half of these co-transcripts displayed tissue-specific expressions. Furthermore, 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. However, the intergenic distance was contributive to co-transcription but not significantly. Our results indicate that co-transcription and intergenic splicing are widely spread perhaps as a specific mechanism to increase the transcription complexity in mammals. Moreover, proteins in multi-component complex or sharing one pathway appear to produce fused transcripts, indicating possibly more cooperative regulation for their functions.

S12-1:

GENOMIC DISORDERS: THE GENOMIC BASIS OF DISEASE

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Whereas Watson-Crick DNA base pair changes have long been recognized as a mechanism for mutations, rearrangements of the human genome including deletions, duplications, and inversions have been appreciated only more recently as a significant source for genetic variation. Diseases that result from DNA rearrangements have been referred to as genomic disorders. Rearrangements of our genome can be responsible for inherited as well as sporadic traits. The analyses of breakpoints in the proximal short arm of chromosome 17 (17p) reveal nonallelic homologous recombination (NAHR) as a major mechanism for recurrent rearrangements. Genome architectural features consisting of low-copy repeats (LCRs), also called segmental duplications, can stimulate and mediate NAHR. There are positional hotspots for the crossovers within the LCRs. Whereas nonhomologous end-joining (NHEJ) can be responsible for some of the non-recurrent rearrangements many appear to occur by a DNA replication mechanism termed FoSTeS for Fork Stalling and Template Switching. Genomic rearrangements may cause Mendelian diseases, complex traits such as behaviors, or represent benign polymorphic variations. The latter copy number variations (CNVs) are ubiquitous in the human genome. In fact, any two humans contain more base-pair differences due to structural variation of the genome than resulting from single nucleotide polymorphism (SNPs). The mechanisms by which rearrangements convey phenotypes are diverse and include gene dosage, position effects, unmasking of coding region mutations (cSNPs) or other functional SNPs, creating gain-of-function fusion genes at the breakpoints, and perhaps through effects of transvection. De novo genomic rearrangements have been shown to cause both chromosomal and Mendelian disease, as well as sporadic traits, but our understanding of the extent to which genomic rearrangements, gene CNV, and/or gene dosage alterations are responsible for common and complex traits remains rudimentary.

1. Lupski, J.R. and Stankiewicz, P. (2005). Molecular mechanisms for rearrangements and their conveyed phenotypes in genomic disorders. PLoS Genetics. 1:627-633.
2. Lee, J., Carvalho, C.M.B., Lupski, J.R. (2007). A DNA replication mechanism for generating non-recurrent rearrangements associated with genomic disorders. Cell 131:1235-1247.
3. Lupski, J.R. (2007). Structural variation in the human genome. N. Engl. J. Med. 356:1169-1171.
4. Lupski, J.R. (2007). Genomic rearrangements and sporadic disease. Nature Genetics 39:S43-S47.

S12-2:

IDENTIFY NEW GENOMIC DISORDER/SYNDROME BY HIGH-RESOLUTION WHOLE GENOME MICROARRAY-KARYOTYPING

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Large genomic imbalance was found in many defined microdeletion/ microduplication syndromes associated with congenital malformation and unexplained mental retardation. More recently, smaller recurrent or de novo genomic imbalances were identified in patients associated with developmental disorder/cognitive impairment or other complex diseases, such as nonspecific global developmental delay, unexplained mental retardation, autism spectrum disorders, and other neuropsychiatric disorders. Disorders associated with genomic imbalance (called genomic disorders) poses a great challenge to genetic and genomic medicine. High - resolution whole genome microarray-based comparative genomic hybridization (array CGH) or SNP genotyping (SNP array) are powerful tools for identifying such genomic imbalance not detectable on earlier generation arrays or other molecular cytogenetic means. These tools revolutionize the diagnosis of genomic disorders and moving the human genetics into true microarray-karyotyping era.

This presentation will demonstrate: (1) Both high-resolution whole genome array CGH and SNP array are robust platforms for clinical diagnosis of developmental disorders associated with genomic imbalance. The custom whole genome chip with long oligonucleotide-based array CGH has excellent flexibility in design and ease of manufacturing; feasible upgrading with technological advances and rapidly updating with new research finding; and superior resolution and reproducibility than current genome-wide array; SNP array can detect genomic copy number alteration and LOH simultaneously. (2) Recurrent or de novo microdeletion or microduplication are common causes of developmental delay and mental retardation. Most recurrent genomic imbalance events are mediated by recombination between segmental duplicated sequences through a mechanism of non-allelic homologous recombination; de novo genomic imbalance events presumably occur due to non-homologous end-joint recombination. (3) Clinical genetic testing of patients with developmental disorders/cognitive impairment using high-resolution whole genome microarray-karyotyping is revealing the clinical importance of ever smaller deletion and duplication such as imbalance at 17q21.3 associated with developmental delay; at 16p11.2 associated with developmental delay, mental retardation, and autism spectrum disorder; at 15q13.2q13.3 associated with developmental delay, mental retardation, and features of autism spectrum disorder or the other neuropsychiatric disorders. These discoveries may lead to early behavioral interventions that could significantly improve the developmental outcome for individuals with such imbalance.

This presentation will also share our experience, in which we used high-resolution whole genome microarray-karyotyping to identify genomic imbalances/rearrangements in the evaluation of over 2500 patients with developmental delay, mental retardation, autism spectrum disorder, and other cognitive impairment or complex diseases.

S12-3:

MICRORNAS AND MENTAL RETARDATION

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Small noncoding RNA guides, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and repeat-associated small interfering RNAs, 21 to 30 nucleotides in length, could shape diverse cellular pathways, from chromosome architecture, development, and growth control, apoptosis to stem cell maintenance. In fact, it has been estimated that miRNAs could regulate as many as one-third of human genes. MiRNAs and the components of the RNAi pathway have been implicated in diverse human diseases. In my presentation, I will discuss our most recent work on both Fragile X syndrome and Rett syndrome, and how the misregulation of the miRNA pathway could contribute the diseases pathogenesis of these two diseases.

S12-4:

MICRORNA-21 (MIR-21) POST-TRANSCRIPTIONALLY DOWNREGULATES ONCOGENE PLAG1 IN DNA MISMATCH REPAIR DEFECTIVE COLORECTAL CANCER

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Colorectal cancers with defective DNA mismatch repair (MMR) have peculiar pathologic and clinical features. Several reports have showed that it would have transcriptional impress, our aim was to characterize the post transcriptional profile of the MMR-deficient colon cancer. A genome-wide miRNA microarray and quantitative real-time PCR were used to identify differentially expressed miRNAs in MMR-deficient CRC cell lines and patients. Eight miRNAs (miR-21, let-7a, miR-200b, miR-200c, miR-145, miR-127, miR-155 and miR-133b) were identified differently both in cells and tissues. Several of these miRNAs were previously found deregulated in other human cancers. MiR-21, which has been described as an anti-apoptotic factor in human diseases, was found up-regulated in all MMR-deficient cell lines and tissues. We identified the target genes of miR-21 for further study. Our research showed that an inverse correlation between miR-21 and PLAG1, RP2 expression. Meanwhile, the 3'-UTR of the two genes are functional targets of miR-21. These results denote that PLAG1 and RP2 are target genes of miR-21 and might involved in formation of MMR-deficient CRC characteristic.

S12-5:

P53-REGULATED MIR-17-92 MODULATE HIF1A EXPRESSION UNDER HYPOXIA

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MicroRNAs (miRNAs) are 21–23 nucleotide RNA molecules that regulate the stability or translational efficiency of target messenger RNAs. miRNAs have diverse functions, including the regulation of cellular differentiation, proliferation and apoptosis. Although strict tissue- and developmental-stage-specific expression is critical for appropriate miRNA function, mammalian transcription factors that regulate miRNAs have not yet been identified. P53, a tumor suppressor and a transcription factor, has been shown to transcriptionally activate the expression of a number of important genes involved in the regulation of cell growth, DNA damage, angiogenesis, and apoptosis. It is widely accepted that p53 accumulates under conditions of severe/prolonged hypoxia, and activates feed-back mechanisms to decrease HIF-1*α* protein amount. However, the mechanism(s) underlying the repression remains unclear. Here we show that p53 activates expression of a cluster of six miRNAs on human chromosome 13. Chromatin immunoprecipation experiments show that p53 binds directly to this locus. We find that expression of Hif1A is negatively regulated by two miRNAs in this cluster, miR-17-5p and miR-18a. These findings expand the known classes of transcripts within the p53 target gene network, and reveal a mechanism through which p53 simultaneously activates hif1A transcription and limits its translation.

S12-6:

THE *PIR* GENE POLYMORPHISMS ARE ASSOCIATED WITH BONE MINERAL DENSITY IN CHINESE WOMEN

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Up to 80% of variance in bone mineral density (BMD) is genetically determined. Two recent QTL mapping studies in mice located a common locus on chromosome-X, corresponding to a syntenic locus on human chromosome-Xp22. Fine mapping by association in post-menopausal women showed association between lumbar spine BMD and *PIR* gene. We sought to examine the effects of *PIR* gene polymorphisms on BMD in the Chinese population. METHODS: Two cohorts of 2000 men and 2000 post-menopausal women aged 55 - 85 were investigated. BMD at multiple sites was measured by DEXA. 4 tagging SNPs is defined using pairwise R2>0.8 as cutoff to cover the gene and adjacent 10kb regions. RESULTS & DISCUSSIONS: In the women cohort, the tagging SNP rs5935970 showed significant association with BMD at lumbar spine (p=0.01), hip (p=0.006) and whole body (p=0.01) after adjustment for age and BMI. Another tagging SNP located at 3' end (rs4830530) also showed association with BMD at lumbar spine (p=0.002) and whole body (p=0.001). However, there was no association in men cohort. This study replicates the previous association and for the first time demonstrated a generalized effect of the gene on BMD at multiple sites. The association was confined to the female population, which may be related to a fluctuating iron status during reproductive period. Therefore, pirin may be the link between body iron/heavy metal and intracellular signal pathway in osteogenic cells. It is also an indicator showing the importance of sex and genetic interaction on disease predisposition and complex traits.

S12-7:

RECONSTRUCTION OF EPIGENETIC GENE REGULATORY NETWORKS FROM LEUKEMIA MICROARRAY PROFILES

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The epigenetic events have often been observed in cancer, but the underline molecular regulatory network and its roles in cancer development are still unclear. From microarray expression profiles, this research innovatively reverses the epigenetic gene regulatory networks that are associated with certain clinical conditions.

We provide a novel algorithm to identify genes that consistently differentially express between conditions and highly co-express within conditions. It uses the full range of the data instead of relying on discretizations of statistics, and simplifies the way of choosing arbitrary statistical significance thresholds. Beginning with the expression levels of 133 epigenetic genes, statistic results on expression levels are: a) A gene-condition network of 37 genes which are significantly “correlated” to sub-classes of leukemia, by comparing the ranking of gene lists of correlated expression and differentially expression. b) A gene regulation network of 33 genes which are significantly pair-wised interact on direct transcriptional levels in acute lymphoblastic leukemia cells, using mutual information theory which can eliminate the majority of indirect interactions inferred by co-expression methods. c) 16 genes are identified in both above networks, and might play key roles for epigenetic regulation corresponding to subtypes of leukemia.

In the identified networks of gene-condition, some are known disease-specific genes; and in the genes regulation network, several direct transcriptional intersections are recently reported. These candidate genes might not be called as significant by an arbitrary threshold of differential expression, but significant coincidently co-express. Moreover, our method is supplementary to current methods of reconstructing regulatory networks for triplets or more complex of interacting genes. This study should enhance our ability to use microarray data to elucidate functional mechanisms that underlie epigenetic regulation and to identify molecular targets of pharmacological compounds for leukemia treatment.

S12-8:

GENOMIC IMBALANCES IDENTIFIED IN CHINESE PATIENTS ASSOCIATED WITH MENTAL RETARDATION USING AFFYMETRIX 6.0 SNP ARRAY

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** Equally contributed. # Senior author of each institute for the collaboration.*

Genomic imbalances constitute a significant genetic etiology for idiopathic mental retardation, which affect approximately 2-3% of world population, where the prevalence varies from country to country and region to region. Traditional karyotyping and molecular cytogenetic analysis were able to identify large chromosomal aberrations in about 5% of patients with mental retardation. Recently application of microarrays, especially with high resolution whole genome array, substantially increased the power for detecting clinical relevant genomic imbalances; the detection rate has been improved to 10-15% in the studies of patients with mental retardation.

Here we employed the latest SNP array from Affymetrix (SNP array 6.0 with approximately 1.8 millions probes) for interrogating a cohort of 150 Chinese children with idiopathic mental retardation. These patients were systematically evaluated by a team of clinical geneticists and neuropediatricians, in whom common causes of mental retardation including chromosomal abnormality, subtelomeric deletion/duplication, and Fragile X syndrome had been excluded by karyotyping, MLPA testing and Southern blot analysis. In addition, we used 150 ethnicity-matched control sample data for evaluating the detected copy number variants in the patients. We identified clinically relevant genomic imbalances with the size range from 300Kb to 10.8Mb and the detection rate is approximately 18.75%. The examples of detected genomic imbalances (recurrent or de novo) include two cases involving Xq28 duplication involving MECP2 gene, two cases with 22q13 deletion involving SHANK3 gene, one with duplication of William syndrome region on 7q11.23, two cases with interstitial deletion involving the long arms of chromosome 6 and 18 respectively, one case with deletion of typical Prader-Willi syndrome critical region (15q11-q13). To our knowledge, this study represents the first report to delineate the genomic basis of mental retardation in Chinese patients using high resolution SNP array, the findings from this cohort uncovered the diverse genomic imbalances as a frequent primary cause of mental retardation in Chinese patients, much like those were observed in other populations. We further demonstrated the great utility of using SNP array for detecting genomic imbalance.

S13-1:

EPIGENETICS MEDIATES GENE AND ENVIRONMENT INTERACTION

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Epigenetics is defined as heritable changes in gene expression that do not alter DNA sequence but are mitotically and transgenerationally inheritable. The main epigenetic mediators are histone modification, DNA methylation, and non-coding RNAs. They regulate crucial cellular functions such as transcript stability, protein translation levels, genome stability, X-chromosome inactivation, gene imprinting, and reprogramming of non-imprinting genes. Animal studies and epidemiological findings now show that "epigenetic programming" of susceptible genes works on developmental plasticity such that exposures to endogenous or exogenous factors during critical periods permanently alter functional capacities of specific organ systems. In human evolution, such changes are designed to allow the fetus to get a head-start by establishing "adaptive" phenotypes in anticipation of demands of the later-life environment. If the resulting phenotypes match predicted later-life demands the individual will stay healthy whereas a high degree of discordance may increase disease risk. During the past half century of human evolution, we have experienced unprecedented rates of introduction of synthetic chemicals, medical interventions, environmental pollutants, and lifestyle choices. Many of these agents/interventions are now known to result in conflicts with the programmed "adaptive" changes made during early life development, and explain the alarming increases in some human diseases. The recent identification of a growing number of epigenetically regulated genes in various animal model systems has prepared researchers to take on the challenge of characterizing distinct epigenomes related to specific exposures. These epigenomes once validated in human studies could become valuable early biomarkers for detecting exposure-related disorders and to devise measures for their prevention. This talk will discuss how endocrine disruption by estrogen mimics (such as bisphenol A, diethylstilbestrol and genistein) in early life could dysregulate genes linked to cancers through an epigenetic mechanism. It will also present preliminary data on the application of epigenetics to epidemiological studies.

S13-2:

SEARCHING FOR THE TRUE VILLAINS OF LUNG AND LIVER CANCER: FINGER PRINTING DNA DAMAGE IN THE P53 GENE

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The p53 gene is one of the most frequently mutated genes in human cancer; more than 50% of human cancers have mutations distributed in more than 200 sites of this gene, and the mutational patterns found in this gene vary significantly among different cancers. These results indicate that not only does the p53 gene play a crucial role in carcinogenesis but also that the p53 mutational pattern may be determined by the DNA damage pattern induced by different cancer etiological agents. To test these possibilities, we developed a method to fingerprint the DNA damage in the p53 gene at the nucleotide level using the UvrABC incision method in combination with the ligation-mediated PCR technique. We found that: 1) the DNA damage pattern induced by polycyclic aromatic hydrocarbons, strong carcinogens in tobacco smoke, in the p53 gene coincides with the mutational pattern in this gene in lung cancer; 2) all of the carcinogen preferential binding sites in the p53 gene contain a CpG sequence, and this preferential DNA binding is due to C5 cytosine methylation; and 3) DNA damage formed at the p53 mutational hotspots are poorly repaired. We also found that the bladder carcinogen 4-hydroxy-aminobiphenyl preferentially forms DNA adducts at the p53 mutational hotspots in bladder cancer. These results strongly suggest that the p53 mutational patterns in different cancers are mainly determined by the DNA damage pattern induced by cancer etiological agents. We also recently found that: 1) alfatoxin B1 (AFB1)-induced DNA adducts preferentially occur at codon 249 of the p53 gene, the mutation hotspot in AFB1-related liver cancer, and 2) the acrolein (Acr) binding pattern in the p53 gene coincides with the p53 mutation pattern. Epidemiological studies have shown that Chinese cooking fumes to be associated with female lung cancer, and Acr is abundant in cooking fumes. Together these results lead us to hypothesize that Acr is the major lung cancer etiological agent in Chinese women.

S13-3:

ENVIRONMENT, NUTRITION AND METABOLISM AND THE RISK OF NEURAL TUBE DEFECTS IN A HIGH-RISK AREA OF CHINA

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Background: Shanxi Province has historically reported a high prevalence of neural tube defects (NTDs). **Methods:** In order to explore environment, nutrition and metabolism and the risk of NTDs in a high-risk area of China, we performed a case-control study and dietary survey in Luliang mountain area of Shanxi Province from January 1, 2002 to December 31, 2005. By using Chemiluminescent Immunoenzyme Assay, fluorescent polarization immuno assay and high-performance liquid chromatography tandem mass spectrometry and routine chemiluminescent assay, serum concentration of histidine and metabolites which are involved in one-carbon metabolism, such as folic acid, homocysteine, S-adenosylmethionine (SAM), and vitamin B12 in NTDs pregnancies and matched controls were measured. **Results**: We found that the local average nutrient intake was lower than the national average level. In women of childbearing age, the intake of nutrients was much lower than the recommended nutrient intake (9-77%). NTD-affected pregnant women had serum vitamin B_{12} and folate concentrations that were lower than in controls ($p<0.01$). Serum total homocysteine was higher in NTDs group than in control at <21 gestational week ($p<0.01$). Simultaneously, a significantly lower serum concentrations of 5-MeTHF ($P<0.001$), 5-FoTHF ($P = 0.002$), total folate ($P<0.001$) and remarkably higher concentration of SAH ($P = 0.018$) were found in cases compared to that in controls. **Conclusions:** Disturbed one-carbon metabolism is related to increased risk of NTDs in high risk populations. Insufficient intake of some nutrients may be an important risk factor for the high prevalence of NTDs. A dietary supplement, combining folate and vitamin B_{12} might be an effective measure to decrease the NTDs incidence in these areas.

S13-4:

THE HUMAN E-CADHERIN (CDH1) AND TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) GENETIC POLYMORPHISM AND CHILDHOOD ASTHMA

Ruey-Hong Wong[1], Jui-Chi Hsu[1], Chu-Ying Peng[1], Ko-Huang Lu[2, 3], Tsai-Nung Kuo Chou[2], Chiao-Wen Li[1], Ying-Shiuan Li[1], Chin-Chun Chen[1], Shao-Chun Wu[1], Chung-Yen Cho[1]

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Cigarette smoke could cause respiratory epithelium inflammation, decrease epithelial-cell adherence, increase detachment, and might contribute to asthma. E-cadherin is responsible for the formation and maintenance of normal architecture and function of epithelial tissues. Transforming growth factor [TGF]-β1 regulates inflammatory states by promoting leucocytes differentiation and inhibiting T lymphocyte proliferation. Therefore we investigated the associations between CDH1 and TGF-β1 polymorphic variants and risks of childhood asthma. A total of 119 cases and 238 controls were included in the hospital-based case-control study. Questionnaires were administered to obtain demographic data. Environmental tobacco smoke (ETS) exposure indicated number of cigarettes smoked daily from subject's parents at home. Allergen test was performed by intracutaneous skin test or Multiple Antigen Simultaneous Test with Taiwan common aeroallergens. Polymerase chain reactions were conducted for CDH1 and TGF-β1 genetic polymorphisms. Our results showed that higher parental education level, family history of asthma, textile work at home, allergen test-positive, CDH1 AA/CA genotypes, and TGF-β1 TT/CT genotypes were significantly associated with risks of asthma. Compared to allergen test-negative children with CDH1 CC genotype, allergen test-positive children possessing CDH1 AA/CA genotypes had a 19.9-fold risk of asthma (95%C.I.=4.6-87.0). Similarly, children carrying TGF-β1 TT/CT genotypes had higher risk for asthma development. Subsequently, we evaluated combined effects of CDH1 and TGF-β1 genotypes with ETS to childhood asthma. Among allergen test-negative children, those exposed to ≤5 cigarettes daily and bearing 0-1 susceptible genotype were used as reference; elevated risks were observed in those exposed to ≤5 cigarettes daily and bearing 2 susceptible genotypes (RR_m=7.3, 95%C.I.=0.7-71.6) and in individuals exposed to >5 cigarettes daily and bearing 2 susceptible genotypes (RR_m=15.9, 95%C.I.=1.3-189.1), respectively. Therefore, CDH1 and TGF-β1 susceptible genotypes may modulate the development of childhood asthma induced by allergen and ETS exposure.

S13-5:

ABNORMAL BIRTH OUTCOMES AND TOXIC HEAVY METAL EXPOSURE IN AN ELECTRONIC WASTE RECYCLING TOWN OF CHINA

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Objectives: To investigate abnormal birth rate of newborns in an e-waste recycling town, Guiyu, and to explore the relationship between neonate's health and exposure to lead, cadmium and chromium. **Methods:** Retrospective study on neonates' birth in Guiyu and Xiamen from 2003 to 2007 was performed. Two hundred and eighty nine neonates from Guiyu and 134 neonates from neighboring town were selected in this study. The lead/chrome/cadmium levels in umbilical cord blood were determined with atomic absorption spectrophotometry. Questionnaires related to the exposures were administrated to lying-in women. Restriction fragment length polymorphism (RFLP) was applied to analyze ALAD gene polymorphism. Comet Experiment was used to examine lymphocyte DNA damage. **Results:** The premature birth, dead fetus, twins and low birth weight rate in Guiyu were all higher than Xiamen. Compared with the control group, neonates in Guiyu had significantly higher levels of lead, cadmium and chrome in umbilical cord blood ($P < 0.01$), and mean lead level in 2004-2005, 2006 and 2007 were 122.28, 113.28, 110.86 μg/L respectively, mean cadmium level were 5.86, 5.30, 3.47 μg/L respectively. The relatively high levels of lead and cadmium in neonates were found correlate with their parents' engagement relating to e-waste recycling. The chrome levels in umbilical cord blood in Guiyu neonates were 303.38 μg/L and 99.90 μg/L in 2006 and 2007 respectively. Lead sensitivity gene ALAD genetic polymorphism of newborns in Guiyu was not correlated with umbilical cord blood Lead level. There were remarkable differences of tailing rate and tail long between Guiyu Group and Control Group ($P<0.05$), and correlations between DNA damage and umbilical cord blood chromium and cadmium levels of newborns ($P < 0.05$). **Conclusions**: Primitive and unregulated e-waste processing in Guiyu had caused high lead, cadmium, and chrome exposures and influenced the health of local neonates.

S14-1:

GENETICS/GENOMICS STUDIES OF OSTEOPOROSIS

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Osteoporosis is a disease characterized by fragile bones and high susceptibility to low-trauma fractures. It is a serious health problem, especially in elderly women. Bone mineral density (BMD) is a major risk factor for defining and studying osteoporosis. BMD has high genetic determination, with heritability ranging from 50 to 90%. Various gene mapping approaches have been applied to identify specific genes underlying osteoporosis, largely using BMD as the study phenotype. We review here the genetic determination of osteoporosis as defined by BMD and discuss a fundamental issue we encounter in genetic research in osteoporosis: the choice of phenotype(s) to study. We briefly summarize and discuss advantages and disadvantages of various approaches used in genetic studies of osteoporosis. We review and discuss the current status for mapping and identification of genes for osteoporosis, including many studies from our own group for genetic epidemiology studies at DNA level, DNA microarray studies at transcription level, proteomics studies at proteome level and some molecular mechanism studies of novel genes we identified. We also introduce our work in bioinformatics involved in our genetics/genomics studies highlighting the importance of data analyses and management in dealing with high-throughput data.

S14-2:

ANALYSIS OF GENOMIC ADMIXTURE AND SELECTION OF INFORMATIVE MARKERS FOR MAPPING DISEASES IN UYGHUR

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Uyghur in Xinjiang of China (UIG) is a population presenting a typical admixture of eastern and western anthropometric traits, we dissected its genomic structure at population level, individual level and chromosome level using 20,177 SNPs spanning over almost entire chromosome 21. Our results showed UIG was formed by two-way admixture with 60% of ancestry from European and 40% of ancestry from East-Asian. Overall linkage disequilibrium (LD) in UIG was similar to that in its parental populations represented by East-Asian and European for common alleles, and UIG manifested elevation of LD only within 500 kb and at level of $0.1 < r^2 < 0.8$ when ancestry informative markers (AIMs) were used. The size of chromosomal segments that were derived from East-Asian and European were on average 2.4 cM and 4.1 cM, respectively. Both the magnitude of LD and fragmentary ancestral chromosome segments indicated a long period of history of Uyghur. Under the assumption of a hybrid isolation (HI) model, the admixture event of UIG was estimated to have taken place about 126 [107~146] generations or 2520 [2140~2920] years ago assuming 20 years per generation. In spite of long history and short LD of Uyghur compared with recent admixture populations such as African American, we suggested mapping by admixture LD (MALD) is still applicable in Uyghur except about 10-fold AIMs are necessary for a whole-genome scan.

S14-3:

EXPANDED NEONATAL SCREENING IN SHANGHAI

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Inborn Errors of Metabolism (IEM) has a diverse spectrum in different country with different incidence, prompt recognition, diagnosis and treatment is important and the patient could benefit from the early, presymptomatic diagnosis and treatment. Neonatal screening in China began in Shanghai from 1981 and it was first city implement neonatal screening program in 1990. Actually 4 metabolic diseases including PKU, CH, CAH and G6PD were in the list of program of Shanghai with a coverage of 98%, although nationwide neonatal screening program was focused only on PKU and CH with a coverage about 25%. Phenylketonuria is the most common metabolism disorder with an incidence of 1:11,000 in China. Among our 223 HPA patients detected by neonatal screening, 93 patients (86.5%) with phenylalanine hydroxylase deficiency, and 30 patients (13.5%) as tetrahydrobiopterin deficiency, including 6-pyruvoyl-tetrahydropterin synthase(PTPS) deficiency and one dihydropteridine reductase.

The neonatal screening using tandem mass spectrometry was started from 2003 in Shanghai, until end of 2007, about 120,000 neonatal samples were analyzed, 21 cases were confirmed including 6 kinds diseases: PKU/hyperphenylalaninemia, maple syrup urine disease, methylmalonic acidemia, propionic academia, 3-methylcrotonyl-CoA carboxylase deficiency and 2 short chain acyl-CoA dehydrogenase deficiency. The pilot study shown that the inborn errors of metabolism in newborn were 1 ： 5,800 in Shanghai area. Lysosomal storage diseases are affecting many organ systems. In recent few years, more than 40 LSD patients, including mucopolysaccharidosis (MPS) type I, MPS□, MPS □A, MPS □ GM1 were diagnosed in our clinical using enzyme activity measurement or molecular biology technique. The expansion of lysosomal storage diseases in the list of neonatal screening might be further considered.

S14-4:

CHINESE HUMAN GENOME DIVERSITY: CLUES ON ADAPTIVE SELECTION?

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There are 56 ethnic minorities in China. The ethnic origin and genetic phenotypes, each minority group has its unique characteristics. There are significant differences in RBC enzymes, HLA antigens and the type and incidences of genetic diseases.

Human mitochondrial DNA (mtDNA), the extra-nuclear genetic material, is characterized by high copy numbers, lack of recombination, high mutation rates and inheritance through purely maternal lines. All these characteristics offer the potential of investigating human origin and evolution. Previous studies indicated that mtDNA sequences differ significantly among different geographical regions. This was traditionally attributed to the hypothesis of genetic drift. However, the effect of selection on characteristic variation in mtDNA has not yet been proved.

Recently, we focused on Tibetans, who for generations have resided in Tibet, and Han Chinese populations from Beijing to compare the mtDNA whole sequences between the two populations. mtDNA sequences were determined using an Applied Biosystems 3730 DNA Analyzer. Data was analyzed using softwares phredPhrap 16.0, Network, DnaSP 4.20.2 and SPSS 15.0. Each SSPro and SSPro8 softwares were used to predict the protein secondary structure while the 3Dpro software was used to calculate the alpha carbon atom coordinate in PDB format. The MaxSprout was used to fill the coordinates of other atoms while Rasmol and Swiss-pdbviewer softwares were adopted to compare the structure. The structure changes of RNA due to mutation were analyzed using the RNAfold software.

Our results showed that the 90 pooled subjects pertained to the Macrohaplogroup M and N, and were classified into 13 haplogroups. No difference was observed among all haplogroups between the two populations except for the M9 haplogroup. Principal component analysis indicated that the first and second principal components (PC1 and PC2) accounted for 41.3% and 10.7% of the total variance, respectively, with the added contribution of 52.0%.

Further analysis indicated that the Tibetan and Han populations pitched different quadrants, and PC1 and PC2 respectively accounted for 77.1% and 10.1% of the total variance with a total contribution of 87.2%, suggesting obvious geographic differences. A total of 18 variants were detected by comparing the mtDNA whole sequences between Tibetan and Han populations. Of that total, 5 variants were unreported and 8 variants were defined at the internal branch.

The analyses of the mtDNA whole sequences provide clues for the existence of adaptive selection for ATP6, ATP8, cyt B, ND2 (G4491A), CO2 (G7697A), tRNA alanine (T5628C) and 12S rRNA (A1041G) genes. This selection might be attributed to the special geographic environment such as high-altitude, hypoxia, extreme cold, etc.

S14-5:

PREDICTING THE NUMBER AND SIZES OF IBD REGIONS AMONG FAMILY MEMBERS AND EVALUATING THE FAMILY SIZE REQUIREMENT FOR LINKAGE STUDIES

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With genotyping of high-density single nucleotide polymorphisms (SNPs) replacing that of microsatellite markers in linkage studies, it becomes possible to accurately determine the genomic regions shared identity by descent (IBD) by family members, without consideration of recombination fractions. In addition to evaluating the likelihood of linkage for a region with the underlining disease (the LOD score approach), an appropriate question to ask is what would be the expected number and sizes of IBD regions among the affected, since there could be more than one region reaching the maximum achievable LOD score for a given family. Here we introduce a computer program to allow the prediction of the total number of IBD regions expected from linkage studies and their sizes. Reversely, it can be used to predict the portion of the genome that can be excluded according to the family size and user-defined inheritance mode and penetrance. Such information has implications on the feasibility of conducting linkage analysis on a given family of certain size and structure or on a few small families when inter-family homogeneity can be assumed. It can also determine the most relevant members to be genotyped for such a study. Simulation results showed that the IBD regions containing true mutations are usually larger than regions IBD due to random chance. We have made use of this feature in our program to allow evaluation of the identified IBD regions based on a Bayesian method and simulations. Our analysis indicates linkage studies may be done on families not considered of sufficient size by classical views.

S14-6:

POSITIVE ASSOCIATION OF SCHIZOPHRENIA TO THE JARID2 GENE REVEALED IN A WHOLE GENOME SCAN IN A HOMOGENOUS POPULATION IN SHANDONG PENINSULA OF CHINA

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DNA pooling can provide an economic and efficient way to detect susceptibility loci to complex diseases. We carried out a genome screen with 400 microsatellite markers spaced at approximately 10 cM in two DNA pools consisting of 119 schizophrenia (SZ) patients and 119 controls recruited from a homogenous population in the Chang Le area of the Shandong peninsula of China. Association of D6S289, a dinucleotide repeat polymorphism in the JARID2 gene with SZ was found and confirmed by individual genotyping (X^2 = 17.89; P = 0.047). In order to refine the signal, we genotyped 14 single nucleotide polymorphisms (SNPs), covering JARID2 and the neighboring gene, DNTBP1, in an extended sample of 309 cases and 309 controls from Shandong peninsular (including the samples from the pools). rs2235258 and rs9654600 in JARID2 showed association in allelic, genotypic and haplotype tests with SZ patients from Chang Le area. This was not replicated in the extended sample. We conclude that JARID2 could be a susceptibility gene for SZ.

S15-1:

UNRAVELING THE GENETICS OF CONGENITAL DISORDERS

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Congenital disorders is a broad category that includes a variety of conditions ranging from minor physical anomalies, severe malformations of single systems to combinations of abnormalities affecting several parts of the body. While a few congenital diseases are caused by single gene mutations, the majority of these disorders are caused by complex interactions between genes and the environment and as such should be considered as multifactorial or complex diseases. Finding the genes underlying these conditions is paramount to the disease management. I will discuss the recent developments on Hirschsprung's disease (colon aganglionosis) to illustrate the current approaches taken to uncover the genetic basis of these diseases. I will also be presenting the current status on the genetic research of other congenital disorders such as biliary atresia and anorectal malformations.

Colon aganglionosis is attributed either to a failure of the enteric neurons precursors (ENPs) to populate the gut or to the demise of such precursors in the distal intestine. The latter is based on the abnormalities of components required for neuronal growth in the aganglionic gut environment. However, the ENPs integrity is also relevant. In this regard, the major findings relate to the mutations found in genes that encode receptors to the signaling molecules present in the intestinal environment. It remains unknown whether the abnormalities observed in the aganglionic gut are the cause or the result of the aganglionosis. The colonization of the colon is regulated by specific signals from both within ENPs and the intestinal environment. The success of the gut colonization depends on the synchronization and balance of the signaling network implicated. DNA alterations in the genes encoding signaling molecules may interfere with the colonization process, and consequently represent a primary etiology for HSCR. The large range of interactions that take place during development is reflected in the variability of the HSCR phenotype, which may result from mutations in a major gene encoding a crucial molecule or from accumulation of less severe mutations in several genes. HSCR is probably the best example of an oligogenic disease, whose components are currently being revealed.

S15-2:

PREDICTION AND PREVENTION STRARTEGIES FOR TYPE 1 DIABETES BASED ON GENETIC, GENOMIC, PROTEOMIC AND ENVIRONMENTAL FACTORS

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Type 1 diabetes (T1D) is an autoimmune disorder characterized by the immune destruction of the insulin producing β cells of the pancreatic islets. Autoimmunity towards pancreatic antigens results from complex interactions between multiple genes, environmental factors and the immune system. The autoimmune process may occur many years before the onset of clinical diabetes and this long asymptomatic period provides excellent opportunities for the prediction and prevention of the disease. Research in past four decades has identified a number of risk factors including susceptibility genes, gene and protein expression changes, cellular changes as well as environmental triggers, which may serve as excellent biomarkers for risk assessment. Despite the identification of multiple useful biomarkers, the existing tests for T1D prediction are still imperfect and earlier biomarkers are also urgently needed. I will discuss recent progress in biomarker discovery using high throughput genetic, genomic and proteomic technologies as well as modern computational technologies. Furthermore, I will discuss recent advances in developing intervention strategies for the prevention of the disease based on immune regulation.

S15-3:

FORWARD GENETIC SCREEN FOR MUTATIONS IN MICE CAUSING CONGENITAL HEART DISEASE

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To elucidate the genetic basis for congenital heart disease (CHD), we carried out a forward genetic screen with ENU mutagenesis in mice to recover recessive mutations causing CHD. Mice like humans have left-right asymmetric 4-chamber hearts with separate pulmonary and systemic circulation, structures that are major targets of CHD. Using noninvasive fetal echocardiography, a high throughput cardiovascular phenotyping protocol was established and over 13,000 C57BL6/J mouse fetuses were ultrasound scanned. These fetuses were derived from 477 G1 males, with an estimate of 50% genome coverage. Cardiovascular defects were identified in 541 fetuses, including most of the major CHD found clinically, such as outflow and atrial/ventricular septation defects, single ventricle and other chamber defects, and aortic arch and venous anomalies. Mutants were intercrossed into the C3H strain background and using B6/C3H polymorphic DNA markers, 15 of the mutations were mapped. All 15 mutants exhibited marked genetic modifier effects, a problem that was effectively managed by intercrossing with B6/AJ consomic mice for rapid recovery of the C57BL6J genetic background. Four of the mutations caused left-right patterning defects in conjunction with CHD. Seven caused CHD together with limb anomalies. Using a combination of strategies including exon and cDNA sequencing, microarray gene expression profiling, and massively parallel sequencing of BAC contigs spanning the mutation interval, 11 mutations were identified. Four were missense, one nonsense, four splicing defect, and two insertion/deletion mutations. Many of the mutations corresponded to genes that were not previously known to cause CHD. Significantly, several of the mutations were shown to cause defects involving the cilia, with altered sonic hedgehog signaling indicated in some of the mutants. The results of this forward genetic screen suggest CHD is acutely sensitive to genetic modifier effects, with the cilia playing an important role in developmental processes integral to structural heart disease.

S15-4:

ONE-CARBON METABOLISM AND BREAST CANCER: A PATHWAY-BASED APPROACH

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Breast cancer is a manifestation of abnormal genetic as well as epigenetic changes. Global hypomethylation, accompanied by promoter hypermethylation, is a common feature of breast tumor cells. Global hypomethylation is thought to induce chromosomal instability, reactivate transposons, promote loss of imprinting, and activate proto-oncogenes. Promoter hypermethylation, on the other hand, appears to be associated with inactivation of genes in virtually all pathways protective of carcinogenesis (e.g. DNA repair, cell cycle control, inflammatory/stress response, detoxification, apoptosis, etc.) including those of breast cancer.

One-carbon metabolism facilitates the cross-talk between genetic and epigenetic processes by playing critical roles in both DNA methylation and DNA synthesis. It provides essential cofactors in the production of primary methyl donors for methylation of DNA, RNA and protein, as well as of dUMP to dTMP in DNA synthesis. A low methyl supply induces DNA global hypomethylation as well as deficient methylation of dUMP to dTMP leading to uracil misincorporation. These processes may result in aberrant DNA repair leading to DNA strand breaks, enhanced mutagenesis and apoptosis. It is important to note that methylation patterns are subject to clonal transmission, and as a result disturbances in one-carbon metabolism can have long-term consequences even after one-carbon metabolism has returned to normal.

This talk will focus on our systematic investigation of the role of one-carbon metabolism in breast cancer etiology and survival. Using a population-based case-control study, Long Island Breast Cancer Study Project, we have examined the inter-relationships among dietary methyl intake, functional polymorphisms related to one-carbon metabolism, and methylation status in tumors. Using structural equation modeling, we have developed a pathway model to study the complex relationship among lifestyle as well as genetic variables involved in one-carbon metabolism. This pathway model is based on a solid understanding of one-carbon metabolism and may provide a way to examine this multifaceted process. We hope to better elucidate one-carbon metabolism's role in breast cancer etiology and provide encouragement for using this innovative method to examine other complex pathways that may play a role in breast cancer development.

S15-5:

LYSYL OXIDASE-LIKE 1 GENE POLYMORPHISMS NOT CONTRIBUTING TO PRIMARY OPEN ANGLE GLAUCOMA

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Purpose: Glaucoma is a multifactorial and polygenetic disease. Recently, single nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (LOXL1) gene, located in locus 15q24.1, have been reported to confer susceptibility to exfoliation glaucoma. This gene is in close proximity to GLC1N (15q22-q24), which is a candidate locus for juvenile-onset primary open angle glaucoma. We conducted our study in order to test for the association between LOXL1 and primary open angle glaucoma (POAG). **Methods:** A total of 292 unrelated POAG patients along with 196 ethnically matched normal controls were recruited from the Hong Kong Chinese population. rs2165241, rs1048661 and rs3825942 of LOXL1 were screened by TaqMan Assay and confirmed by direct sequencing. Linkage disequilibrium (LD) and haplotype analysis were done with the Haploview software. **Results:** The genotype distribution of the 3 SNPs followed Hardy-Weinberg equilibrium. The G allele in rs1048661, which is reported to be a functional risk allele for exfoliation glaucoma, existed almost in identical frequency in Chinese POAG cases and controls (0.4178 versus 0.4592) (p= 0.201). The frequency of G allele in rs3825942 was 0.8955 vs 0.8724 (p=0.265), while the frequency of C allele in rs2165241 was 0.9161 vs 0.8954 (p=0.273) in case and control respectively. rs1048661 and rs3825942 were in the same LD block, no significant difference was found between the haplotypes generated with these intragenic SNPs with POAG phenotypes. **Conclusions:** We have excluded the involvement of LOXL1 SNPs in POAG in the Hong Kong Chinese population, which is in consistent with previous studies from other populations.

S15-6:

PROGRESS ON ETIOLOGICAL STUDY OF CONGENITAL HEART DISEASE AND STRATEGY OF CANDIDATE GENE CLONING

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Congenital heart disease is the most common birth defect, affecting 1% of liveborn infants. Identification of etiology that causes congenital heart disease in human beings is a formidable task. A precise etiological diagnosis can have important implication for treatment and follow-up, but also in counseling on recurrence risks. In this review we overview the genetics and approaches applied in genetic diagnosis of congenital heart disease as well as the way and strategy for further study.

The diseases caused by chromosomal anomalies often occurred with syndrome. The incidence of heart defects and the affected tissues vary in different types of chromosomal disorders. Turner syndrome, 18-trisomy, 21-trisomy and 13-trisomy syndromes are highly accompanied by heart defects. Microdeletion of 22q11, 1p36.4-1pter, 9q34-qter, 8q12, 13q22-qter and 15q11 often shows heart defects. The development of the heart is regulated by many genes. Any aberration of these genes may cause heart defects. Mutation of some genes is related to specific heart defects. At least 40 genes have been reported related to congenital heart diseases, including transcription factors, extracellular matrix conjunction proteins, enzymes as well as position decision genes of internal organs. Heart diseases are also related to the abnormal expression of miRNA.

High occurrence of congenital heart defects occurring in Chromosomal anomalies and dysmorphic syndrome keep karyotype analysis remain the prevalent method for etiological study of this disease. FISH is a fast method to identify aneuploid aberration and chromosomal microdeletions from the interphase nucleus. MLPA and array-MLPA are new screening technique for copy number aberration and mutation of genes. Gene mutation is a major etiology of congenital heart disease, so PCR based mutation test is still a main approach for screening some specific congenital heart diseases. Array Comparative Genomic Hybridization (array-CGH) is a novel technique for high resolution, genome-wide screening for submicroscopic chromosomal imbalances with current cytogenetic techniques. A chromosomal aberration is identified only in a subset of patients suffered from congenital heart defects. This is in part explained by the limited resolution of standard karyotyping, which is unable to detect imbalances smaller than 5-10Mb.

S15-7:

EVALUATION OF 7 MONOGENIC BONE DISEASE GENES REVEALED A STRONG ASSOCIATION OF -301T/C OF *SOST* WITH OSTEOPOROSIS

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We sought to determine whether the allelic variation in 7 monogenic bone disease genes (*CLCN7, TCIRGI, SOST, CA2, CSTK, TGFB1* and *SLC26A2*) contributes to osteoporosis / bone mineral density (BMD) variation in the normal Chinese population. We conducted a gene-wide and tag SNP-based association study in 1, 243 case-control Chinese subjects. Twenty-two tag SNPs from 7 monogenic bone disease genes were selected based on the CHB panel of the Phase II HapMap Project, and genotyped. Allelic and haplotype association analyses were conducted by Haploview and binary logistic regression analyses. Gene–gene interactions were investigated using multifactor dimensionality reduction method. The promoter SNP rs1230399 (-301T/C) of SOST showed significant genotypic and allelic associations with BMD at all skeletal sites measured (P = 0.04-0.001). Importantly, this association has been replicated in the Caucasian population. Functional analysis showed that the rs1230399 was located at the core consensus recognition site of two important transcription factors C/EBPα and FOXA1 which were involved in the Wnt and estrogen signaling pathway. T→C mutation abolishes the binding of both C/EBPα and FOXA1 to SOST. In addition, significant gene-gene interactions were identified for SOST/CLCN7 and TGFB1. In conclusion, the C-allele of -301T>C variant of SOST was associated with high BMD. The variant may mediate BMD by Wnt and estrogen signaling pathways.

S16-1:

RE-INVENTING PCR: APPLICATIONS OF A NOVEL MULTIPLEX PCR TECHNOLOGY

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Personalized medicine starts with a personalized diagnosis. Phenotype based disease classifications are usually too crude to guide personalized treatment. Detailed genotype profiling is usually required for molecular differential diagnosis. However, clinical applications of traditional PCR based methods are limited because difficulties to perform multiplex PCR. We have developed a novel multiplex PCR method called “target enriched multiplex PCR” (tem-PCR) that making highly efficient molecular differential diagnoses possible. With the tem-PCR method, for each target in the multiplex PCR reaction, nested gene-specific primers are designed and included in the reaction. These primers are used at extremely low concentrations and are used only to enrich the targets during the first few cycles of PCR. Some of these gene-specific primers have tag sequences that can be recognized by a universal set of primers, called SuperPrimers™. Only the SuperPrimers™ are included at a concentration necessary for exponential amplification, and only the reverse SuperPrimer™ is labeled. Labeled PCR products are detected with a complimentary capture probe that is covalently coupled to a color-coded bead. Tem-PCR works because it addresses two of the most difficult problems inherent in multiplex PCR: (1) incompatibility of amplification conditions among different primer sets and (2) background amplification associated with high concentrations of primers. Using this technology, we have successfully developed a series of multiplex assays for the diagnosis of various infectious diseases. The method could also be used in other genotype profiling studies for genetic diseases or cancer.

S16-2:

DEVELOPMENT OF DNA ANALYSIS TECHNOLOGIES: ANALYSIS OF mRNA IN A SINGLE-CELL

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Various new technologies have played important roles in the development of life science as well as biotechnology fields. The automation of DNA sequencing processes encouraged the human genome initiative. We have developed capillary array DNA sequencers for genome analysis that contributed to the completion of the human genome sequencing and opened the post genome era. Ultra high throughput DNA sequencers based on pyrosequencing or single DNA molecule sequencing have been developed recently which can reduce the sequencing cost quite a lot. Besides the high throughput analysis of genomes, the analysis of gene expressions is very important for understanding a real life system. As a single-cell is the minimum unit of life, the quantitative analysis of mRNA in a single-cell is getting important. Although DNA chips are used for gene expression analyses, they are not suitable for the accurate quantitative analysis of mRNA in a single-cell. We need new tools for that. So we started a research program called “life surveyor” to develop various tools for analyzing mRNA, proteins and metabolites in a single-cell. A preliminary result as to the analysis of mRNA in a single-cell indicates that gene expression levels change from cell to cell even if the cells are treated equally and the noise levels are much bigger than the experimental errors. New insights about a real life system will be obtained with the new tools for single-cell analysis.

S16-3:

ARRAY-BASED DNA SEQUENCING AND DISEASE INVESTIGATION

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The ability to determine nucleic acid sequences is of utmost importance for the detailed study of biological systems. With the completion draft of the human genome, we are entering a new era in the biological sciences with the DNA sequencing as one of the main catalysts. High throughput and affordable DNA sequencing techniques would undoubtedly continue the stream of the revolution initiated by the Human Genome Project. Recent impressive advances in DNA sequencing technologies have accelerated the detailed analysis of genomes from many organisms. We have been observing numerous reports of complete or draft versions of the genome sequence of several well-studied, multi-cellular organisms. The Human Genome Project was made achievable by a significant reduction in DNA sequencing cost by three orders of magnitude; a further cost reduction of two to three would launch a new era of DNA sequencing applications from short DNA reads to whole genome sequencing. In the DNA sequencing field, Pyrosequencing has emerged as a technology for de novo high throughput whole genome sequencing. The method is based on the principle of sequencing-by-synthesis and pyrophosphate detection through a series of enzymatic reactions to generate luminescence sequence peak signals. Pyrosequencing is being used from single nucleotide to genome sequencing and the method is commercially available for low throughput sequencing by Biotage and high throughput sequencing by 454 Life Sciences (currently owned by Roche). Further developments and improvements could increase the throughput and decrease the cost of sequencing by another two orders of magnitude within the next five years. At Stanford Genome Technology Center, we are developing further array-based high-through Pyrosequencing to minimize the cost and increase the output sequence data as well as employing this technology for disease investigation.

S16-4:

SAMPLE AND ASSAY TECHNOLOGIES FOR MOLECULAR MEDICINE

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Molecular Genetics provides new approaches for the early detection and diagnosis of infectious diseases and cancer. A prerequisite to the establishment of molecular diagnostic testing is integrated and effective management of the `lifestream` of a biological sample from its collection, via stabilization and storage, enabling nucleic acid enrichment and standardized purification of the analyte and ultimately link it to the respective molecular diagnostic assay. We will provide details on each important step of the workflow and discuss examples for tailor made assay development. The development of a new HPV assay will be discussed in detail.

S16-5:

THE DROSOPHILA MODEL FOR SCREENING GENE CANDIDATES RELATED TO HEART FAILURE ON A LARGE SCALE

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Despite recent advances in preventing deaths related to cardiac disorders, cardiovascular disease (CVD) remains the leading cause of death in USA as well as in China. Cardiac arrhythmias and heart failure are common in CVD patients; however, the mechanisms underlying heart failure have remained elusive. Because the basic mechanisms of heart development and function are conserved between Drosophila and vertebrates, we have recently developed an external electrical pacing paradigm to use the fly heart and the power of Drosophila genetics to understand the molecular mechanisms underlying aging of cardiac tissue and their contribution to cardiac disorders and heart failure. In this model, flies are aligned on a specially prepared conductive glass slide and stimulated by a standard square wave stimulator to accelerate their heart rate, which at rest is 3Hz, to 6 Hz for 30 seconds. When the current pulses are turned off, the flies will either recover to regular near normal heartbeat or fibrillate or undergo arrest. Flies which have experienced either fibrillation or arrest will be observed again for a period of 2 min in order to chart the percentage that recover to a normal resting heartbeat within the allotted time frame. This assay provides to examine age-dependent declines in heart function of various genotypes and to conduct large-scale genetic screens for loci that affect cardiac performance in an age-related manner. For example, at 1 week old, wild-type flies have a lower failure rate of 24%, KCNQ mutant flies have a higher failure rate of about 65%. While at 5 weeks old, both wild-type and KCNQ mutant flies have the highest failure rate of 70%-80%. So by refining measures of heart performance in young adults and old adults for high-throughput and combining them in the same screen, one can immediately separate mutations or genes that accelerate or mitigate the age-related decline of adult heart performance. Taking advantage of this model, we screened about 300 deficiency lines which deleted different fragments related to the 1st and the 2nd chromosome of Drosophila, and obtained about 60 deficiencies' candidates, with a higher heart failure rate by themselves or when interacted with other heart genes. These gene candidates and their functions will be further identified and studied.

S16-6:

GENETICALLY MODIFIED RABBIT MODELS FOR MEDICAL SCIENCES

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Genetically modified (GM) rabbits have been proved to be excellent animal models for both inherited and acquired human diseases. GM rabbits expressing human genes have been widely used as models for cardiovascular disease, AIDS, and cancer research. So far, GM rabbits were almost exclusively generated by pronuclear microinjection, which randomly leads to additive genes integrated in the rabbit genome; however, recent progress in gene targeting and somatic cell cloning using nuclear transfer has opened new avenues for production of GM rabbits. RNA interference is also quickly becoming a valuable experimental tool that allows investigators to knock down the expression of specific genes, and makes it possible to create GM rabbit models in the near future. In this meeting, we will review the progress of GM technology in rabbits during the past years and emphasize their applications as a model in studying human disease.

S17-2:

NOVEL PPARγ GENE MODULATION USING AD-PPARγ AND ROSIGLITAZONE AMELIORATES NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: PPARγ ligands improve non-alcoholic steatohepatitis in human. However, the treatment response is highly variable and difficult to predict, which may be associated with PPAR deficiency. We sought to determine the effect of overexpression of PPARγ through gene delivery with or without PPARγ ligand in the development of steatohepatitis. The mechanisms of its action were investigated. **Methods:** Male wild-type or PPARγ+/- mice were fed the MCD diet with or without overexpression of PPARγ delivered by adenovirus (Ad-PPARγ) and/or rosiglitazone (150, 300 ppm), a potent PPARγ ligand. Controls were fed the same diet supplemented with methionine and choline. Liver injury (serum alanine aminotransferase), hepatic inflammation, triglycerides (TG), and lipid peroxide levels were determined. Expressions of adipokines, fatty acid uptake, lipogenic genes in liver and white adipose tissue were assayed. **Results:** Wild-type mice fed the MCD diet developed moderate steatohepatitis. Rosiglitazone ameliorated liver injury. PPARγ+/- mice fed the MCD diet developed more severe steatohepatitis than wild-type mice, and were unaffected by rosiglitazone. PPARγ gene delivery using Ad-PPARγ in wild-type mice increased responsiveness to rosiglitazone and was able to prevent and reverse steatohepatitis. This effect was associated with an increased body weight and increased expression for fatty acid uptake genes (aP2, FATP-1, CD36 and LPL) and lipogenic genes (SREBP-1, SCD-1 and FAS) in adipose tissue and to a lesser extent in liver. The anti-steatohepatitis action of PPARγ was also mediated via regulating adipose tissue-generated adipokines, suppressing TNF-α and IL-6 and inducing adiponectin, thereby increasing hepatic fatty acid beta-oxidation and reducing steatohepatitis. Moreover, PPARγ activation suppressed hepatic lipoperoxide and reduced proinflammatory cytokines (TNF-α and IL-6) production. **Conclusion:** These data suggest that PPARγ is an important endogenous regulator and potential therapeutic target for nutritional steatohepatitis, an effect associated with diversion of fat storage from the liver to the adipose tissue.

S17-3:

GENOTYPE-SPECIFIC GENOMIC MARKERS ASSOCIATED WITH HEPATITIS B VIRUS-RELATED PRIMARY HEPATOCARCINOMA

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Hepatitis B virus (HBV) infection is the major cause of hepatocellular carcinoma (HCC) worldwide. The pathogenesis of HCC due to chronic infection of HBV has been studied extensively and the molecular changes during malignant transformation are analyzed as well. In this project, we aimed to identify genomic markers in hepatitis B virus (HBV) that are associated with HCC development by comparing the complete genomic sequences of HBV among patients with HCC and those without. One hundred patients with HBV-related HCC and 100 age-matched HBV-infected non-HCC patients (controls) were studied. HBV DNA from serum was directly sequenced to study the whole viral genome. To derive predictive algorithm for the tumor development, the HBV genomic data was studied by molecular evolutionary analysis, clustering, feature selection, classifier learning and many other data-mining algorithms. Finally, an independent cohort of 132 cases (43 HCC and 89 non-HCC) was used to validate the accuracy of these algorithms. We found that infections with different genotypes of HBV (B, Ce, and Cs) carry different genomic markers for HCC at different parts of the HBV genome. Different HBV genotypes may have different virologic mechanisms of hepatocarcinogenesis.

S17-4:

HOST GENETIC SUSCEPTIBILITY TO EMERGING INFECTIOUS DISEASES

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In the face of emerging epidemics, understanding variation in individual responses to pathogens takes on new importance. Two closely related trans-membrane C-type lectins DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) and L-SIGN (liver/lymph node-specific ICAM-3 grabbing non-integrin) recognize a wide range of micro-organisms. Both genes encode an extended neck-region consisting of tandem-repeats that support the carbohydrate recognition domain which plays a crucial role in influencing the pathogen-binding properties of these receptors. This neck-repeat region remains relatively constant size for DC-SIGN, but has considerable polymorphism for L-SIGN. Homo-oligomerization of this neck-region is important for high-affinity ligand binding. We have shown that heterozygous expression of L-SIGN in which neck lengths differ can affect ligand-binding affinity. By *in-situ* hybridization, we demonstrated that L-SIGN is expressed in lung in cytokeratin positive alveolar epithelia as well as a subset of cells co-expressing ACE2 but negative for cytokeratin. *In-vitro* experiments showed that L-SIGN binding to SARS-CoV leads to proteasome-dependent viral degradation rather than productive viral replication. Compared with heterozygotes, cells homozygous for L-SIGN show higher binding capacity for SARS-CoV, higher proteasome-dependent viral degradation and a lower capacity for *trans* infection. This was supported by genetic risk association study which showed that individuals homozygous for L-SIGN tandem-repeats are less susceptible to SARS infection. L-SIGN-positive, cytokeratin- and surfactant- negative SARS-infected cells also co-express stem/progenitor cell markers CD34 and Oct-4 which can also be identified in some non-SARS individuals and can be infected *ex-vivo* by SARS-CoV. Worldwide demographic data of the tandem-neck repeat region showing distinct differences in the neck-region allele and genotype distribution among different ethnic groups which support the neck-region as an excellent candidate acting as a functional target for selective pressures exerted by pathogens will be discussed.

S18-1:

TSPY AND ITS X-ENCODED HOMOLOGUE INTERACT WITH CYCLIN B BUT EXTERT CONTRASTING FUNCTIONS ON CYCLIN DEPENDENT KINASE 1 ACTIVITIES

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The testis-specific protein Y-encoded (TSPY) gene is the putative gene for the gonadoblastoma locus on the Y chromosome (GBY). It is tandemly repeated on the Y chromosome, and could be genetically unstable under certain conditions. TSPY is normally expressed in fetal gonocytes and adult spermatogonia in the testis and has been postulated to serve functions in stem germ cell proliferation and meiotic division. It is abundantly expressed in gonadoblastoma, testicular germ cell tumors, and somatic cancers, including prostate cancer, hepatocellular carcinoma and melanoma. TSPY and an X-homologue, TSPX, harbor a conserved domain, designated as SET/NAP domain, but differ at their carboxyl termini. Ectopic expression of TSPY accelerates cell proliferation by abbreviating G2/M stage while over-expression of TSPX retards cells at the same stage of the cell cycle. Previous studies demonstrated that the SET oncoprotein is capable of binding to cyclin B. Using various protein interaction techniques, we demonstrated that TSPY and TSPX indeed bind competitively to cyclin B at their SET/NAP domains. TSPY colocalizes with cyclin B1 at various stages of the cell cycle, particularly on the mitotic spindles at metaphase. TSPY enhances while TSPX represses the cyclin B1-CDK1 phosphorylation activity both in vitro and in vivo. The inhibitory effect of TSPX on the cyclin B1-CDK1 complex has been mapped to its carboxyl acidic domain that is absent in TSPY, suggesting that TSPX could serve a normal function in modulating cell cycle progression at the G2/M stage while TSPY has acquired a specialized function(s) in germ cell renewal and differentiation. Epigenetic dysregulation of TSPY in incompatible germ or somatic cells could promote cell proliferation and predispose susceptible cells to tumorigenesis. Aberrant expression of TSPY in somatic cancers suggests that it could also exert gender-dimorphic effects on the initiation and/or progression of human cancers.

S18-2:

GENETICS OF INTERSEX: ANTAGONISM BETWEEN MALE AND FEMALE SEX-DETERMINING PATHWAYS

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We have performed detailed molecular and cellular analyses of ovotestis development in mice in order to gain insight into the normal sequence of events in mammalian testis determination and the etiology of partial XY sex reversal. In ovotestes of B6 XYPOS fetuses, testicular cords formed only in the central region of the gonad where SOX9 was up-regulated, even though SRY was expressed throughout the length of these gonads. At the poles the ovary-specific protein FOXL2 was expressed. FOXL2-expressing cells also were found among the SOX9-positive pre-Sertoli cells in the central region of B6 XYPOS ovotestes. Our results demonstrate that 1. Both timing and levels of SRY expression are critical for SOX9 expression, and that SOX9, not SRY, is the pivotal determinant of sex differentiation in mice, 2. An active ovarian-determining pathway operates from an early stage in gonadal development, 3. The ovarian pathway is normally suppressed in XY gonads by the action of SOX9, and 4. Compromise of the testis-determining pathway can tip the balance in favour of ovary development.

S18-3:

DEFICIENCY IN THE HNRNP PROTEIN DAZAP1 CAUSES INFERTILITY AND GROWTH RETARDATION IN MICE

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DAZAP1 (Deleted in Azoospermia Associated Protein 1) is a component of the hnRNP particles that is expressed most abundantly in the testis. It first appears abundantly in the nuclei of mid-pachytene spermatocytes, remains in the nuclei of round spermatids, and relocates to the cytoplasm at spermatid elongation. Its exclusion from the transcriptionally inactive XY body in pachytene spermatocyte and its transcription-dependent nuclear localization in somatic cells suggest a role for DAZAP1 in mRNA transcription and transport.

We used a conditional knock-out approach to generate *Dazap1* mutant alleles in order to study the biological role of DAZAP1. Mice homozygous for the null allele are perinatal lethal. Many homozygotes for the hypomorphic floxed allele (Fn) that produced a longer DAZAP1 protein with an insertion encoded by the anti-sense strand of the *neo* gene also died soon after birth. Fibroblasts established from *Dazap1*$^{fn/fn}$ 13.5 day embryos grew poorly in culture. The *Dazap1*$^{fn/fn}$ mice that survived could nonetheless live for more than a year. They had normal appearance but were much smaller in size compared to their wild-type or heterozygous littermates. Both male and female *Dazap1*$^{fn/fn}$ mice were sterile. Males had small testes with atrophic seminiferous tubules that contained a large number of apoptotic cells. There were many germ cells in the tubules, including pachytene spermatocytes with visible XY-bodies in the nuclei. However, both histological and FACS analyses showed the absence of meiotic and haploid germ cells, indicating spermatogenetic arrest right before meiosis. Female *Dazap1*$^{fn/fn}$ mice had smaller ovaries that contained normal appearing follicles at different stage of development. However, mating between female *Dazap1*$^{fn/fn}$ mice with fertile males failed to produce any offspring. In conclusion, the phenotype of our *Dazap1* mutant mice indicates that DAZAP1 is not only essential for spermatogenesis, but also required for normal growth and development of mice.

S18-4:

NEW CANDIDATE GENES FOR "CURLICUE" SPERM MOTILITY ABNORMALITY DISCOVERED BY CGH MICROARRAY

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The motionless spermatozoa, phenotype of *"Curlicue"*, are discovered during the studies of the "*T-complex*" and the "*T haplotype" mouse models*. "*T-complex*" occupies approximately 40-million base pairs (1-40 Mb) of proximal chromosome 17, and exists in natural populations of wild mice of the *Mus musculus* species as a family of homologues called "*T haplotype"* (*t*). *"Curlicue"* is observed in both the heterozygous complete and partial *t/t* equivalents (replacing 29.7Mb-39.7 Mb of *t* with the *Spretus* chromosome, hereafter call S^{R3}/S^{R3} mice) males, which are invariably sterile. The aim of this study is to reveal the molecular basis of *"Curlicue"* by investigating the genomic mutations occurred in the S^{R3}/S^{R3} mice using array comparative genomic hybridization (a-CGH). Sperm motility-related high definition CGH microarray (a total of 175,106 probes) have been designed based on the most updated Agilent database of about 4 million computationally validated CGH probes in this study. Three windows of homozygous deletion have been found covering the chromosome 17 from 36,962,933-37,086,257 bp, 37,277,230-37,329,213 bp and 38,619,976-38,774,571 bp respectively. 5 estimated potential genes (1174, 95281, 1178, 717 and 1192) are located in the deletion areas. Expression of 1174, 95281, 1178 and 717 in the testis was confirmed to be deleted in the S^{R3}/S^{R3} mutated mice by both PCR and RT-PCR. We conclude that the deletion of these genes in the mutated mice might take important roles in the infertility pathology.

S18-5:

PREDICTIVE VALUE OF SEMEN PARAMETERS IN THE MALE CHROMOSOMAL ABNORMALITY

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Karyotype analysis is necessary for ART therapy of male infertile patients and screening of donors. Whether there is a relationship between semen parameters and karyotype abnormality is unknown. In this study, a case-control study was carried out to analyze the value of semen parameters in the male chromosomal abnormality. A total of 1876 male infertile patients (cases) and 2351 healthy donors (control) were examined for karyotype analysis, as well as conventional sperm parameters, notably sperm morphology, sperm count, and progressive motility. The results showed that the rate of chromosome abnormality in male infertile group was significantly higher than that of healthy group. The male infertile patients were further divided into 4 groups, including oligospermia, azoospermia with testicular atrophy, azoospermia with normal testis, asthenospermia and teratozoospermia. The results demonstrated that the rates of chromosomal abnormality were 3.4% (41/1218),46.6% (81/174),8.2% (38/462),9.0% (2/22), respectively, all of which were significant in statistical analysis. The study verified that the chromosomal abnormality is responsible for male infertility, and suggests that semen parameters are predictive for chromosomal abnormality. Low chromosomal abnormal rate in healthy donors suggests that it is not advisable to analyze karyotype of donors before other common items of screening in order to decrease the costs of it.

S18-6:

QUANTITATIVE ABNORMALITIES OF FETAL TROPHOBLAST CELLS IN MATERNAL CIRCULATION IN PREECLAMPSIA

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Objective: To determine if quantitative abnormalities of circulating fetal trophoblast cells (CFTCs) are associated with preeclampsia. **Methods:** The trophoblast cell-specific antibody, MEM-G/9 (monoclonal antibody to HLA-G), was applied to recognize the trophoblast cells from the maternal circulation. The trophoblast cells were isolated by density gradient centrifugation from maternal blood samples of normal pregnant and preeclamptic women, respectively. After preliminary enrichment, the CFTCs were identified by immunocytochemical staining with the MEM-G/9. To prove fetal origin of the HLA-G positive cells, primer extension preamplification (PEP) and polymerase chain reaction (PCR) based on single HLA-G positive cells were adopted to detect human sex-determining region of the Y chromosome (SRY) gene. **Results:** There were 6.88±1.54 and 30.56±5.16 HLA-G positive cells in 6 mL maternal blood from the normal pregnant (n=16) and preeclamptic women (n=18), respectively. The difference was statistically significant ($P < 0.0001$). The SRY gene from the HLA-G positive cells was detected in all pregnant women carrying male fetuses. The sensitivity and specificity of PEP and PCR for the SRY gene detection were 100%. **Conclusion:** It is concluded that enhancement of CFTCs´ numbers is related to preeclampsia, which might be useful for noninvasive prenatal diagnosis of preeclampsia.