P1-1:

THE DIVERSE PHYSIOLOGICAL FUNCTIONS AND PATHOPHYSIOLOGICAL RELEVANCE OF TRANSCRIPTION REPRESSOR ZBTB20

Zhifang Xie[1], Ye Zhang[1], Hai Zhang[1], Michael Grusby[3], Jian Lu[1], Xuetao Cao[2], Weiping Zhang[1,3]

[1]Department of Pathophysiology, [2]Immunology, Second Military Medical University, Shanghai 200433, China; [3]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston 02115, U.S.A.

Transcription repressor is crucial for transcriptional regulation, and plays important roles in mammalian development, physiological homeostasis and pathogenesis. We previously identified a novel Krüppel-like zinc finger protein ZBTB20 (DPZF), which is highly expressed in hippocampus, liver and pancreatic islet. To elucidate its potential physiological functions, we generated its global and conditional knockout mice, respectively. ZBTB20 global knockout mice showed severe growth retardation, high mortality, deafness, infertility, aberrant hair growth, hypoglycemia, and disrupted hippocampus development. Pancreatic cell-specific ZBTB20 knockout mice exhibited diabetes-like phenotype, with severely impaired glucose sensing and insulin secretion. Tissue-specific ablation of ZBTB20 in liver, muscle, or adipose tissues didn't affect glucose homeostasis significantly. Biochemical and functional analysis demonstrated that ZBTB20 is a transcriptional repressor, and alpha-fetoprotein (AFP) is one of its targets. Normally, AFP gene is highly activated in fetal liver, but is dramatically repressed shortly after birth. ZBTB20 ablation in liver led to nearly complete de-repression of AFP gene in entire liver throughout adult life. We demonstrated that ZBTB20 bound to AFP promoter directly and identified the ZBTB20-binding sequence. Our results point to ZBTB20 as a master regulator governing AFP gene transcription in liver. Taken together, ZBTB20 regulates diverse physiological processes, and is potentially relevant in the pathogenesis of cancer, diabetes, and neuronal diseases. (This work is supported by National "973" and "863" projects of China, and NSFC to W.Z.)

P1-2:

GENETIC DIAGNOSIS RESOURCE FOR PRIMARY IMMUNODEFICIENCY DISEASES IN HONG KONG

Koon-Wing Chan[1], Tsz-Leung Lee[1], Tong-Xin Chen[2], Liping Jiang[3], Bee-Wah Lee[4], Xiaochuan Wang[5], Woei-Kang Liew[6], Hsin-Hui Yu[7], Chengrong Li[8], Ah-Moy Tan[9], Lokman Mohd Noh[10], Dawn Li-Chern Lim[4], Mimi L.K. Tang[11], Susanna Fung-Shan Fok[1], Priscilla Hoi-Shan Mak[1], Vivian Wai-Man Chan[1], Marco Hok-Kung Ho[1], Pamela Pui-Wah Lee[1], Xiqiang Yang[2], Yu-Lung Lau[1]

[1]Department of Paediatrics and Adolescent Medicine, Hong Kong Jockey Club Clinical Research Centre, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; [2]Department of Immunology and Oncology, Shanghai Institute for Pediatric Research, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, China; [3]Chongqing Children's Hospital, Chongqing University of Medical Sciences, Chongqing, China; [4]Department of Paediatrics, National University of Singapore, Singapore; [5]Children's Hospital of Fudan University, Shanghai, China; [6]Department of Paediatric Rheumatology/Immunology/Allergy Service, KK Women's & Children's Hospita ,Singapore; [7]Department of Pediatrics, National Taiwan University Hospital, Taiwan; [8]Shenzhen Institute of Paediatrics, Shenzhen, China; [9]Department of Paediatric Haematology/Oncology Service, KK Women's & Children's Hospital, Singapore; [10]Unit of Clinical Immunology, Paediatrics Department, Faculty of Medicine and Health Sciences, University of Putra Malaysia, Malaysia; [11]Department of Allergy and Immunology, Royal Children's Hospita , Australia

Over 150 inherited primary immunodeficiency diseases (PIDs) have been described, with more than 130 causative genes identified due to the recent advances in the gene hunting techniques and molecular diagnostic procedures. Early definite diagnosis of PIDs is important to provide appropriate treatment and management to the patients and the affected families. Till now, the undiagnosed or misdiagnosed of PIDs are not uncommon due to the diversity of phenotypes, the lack of experience and facilities especially in Asia. It is important to raise the national and international awareness on the issue and form networks to facilitate both clinical and molecular diagnoses. In our centre, we aim to provide clinical and genetic diagnoses for suspected PID patients in our region. From 2000-2008, a total of 188 suspected PID patients (68 from Hong Kong, 104 from mainland China, 9 from Singapore, 3 from Taiwan, 3 from Malaysia and 1 from Australia) were referred to us for consultation and investigated

subsequently for genetic diagnosis. 140 mutations were identified in 15 of 31 target genes for XLA, WAS, XCGD, XLP, XHIM, HIGM2, SCID, LAD1, CINCA, IPEX, HIE, ALPS, CVID, HPS, GS2, TRAPS, osteopetrosis and Mendelian susceptibility to Mycobacterial Disease (MSMD). 129 of 193 family members were genetically diagnosed as carriers. Our resources are not only beneficial to the affected families, but also enrich invaluable experience for diagnoses and management of PIDs in individual centre, as well as forming a registry of genetically diagnosed PIDs in Asia.

P1-3:

MOLECULAR CYTOGENETIC STUDY ON FAMILIAL EPILEPSY

SC Chang[1], YL Hsin[2], Ingrid Y Liu[1], JH Yen[1*]

[1]Institute of Human Genetics, Tzu-Chi University, [2]Department of Neurology, Buddhist Tzu-Chi General Hospital , 701, Sec. 3, Chung-Yang Rd., Hualien, Taiwan

Epilepsy is a common but genetically complex neurological disorder that affects 1-2% of world population. Previous studies showed the chromosomal or genetic abnormalities may confer susceptibility to epilepsy, but the genes causing epilepsy have not been well known or characterized to date. In the present study, we reported 6 individuals with epilepsy in the same family, and the clinical work up did not reveal any environmental or infectious factors to cause the disease in this family. The proband was diagnosed as temporal lobe epilepsy (TLE) and we hypothesize genetic factors are major contributed to this familial epilepsy. To investigate the chromosomal aberrations for the familiar epilepsy, chromosome karyotyping and molecular cytogenetic techniques were used for this study. Blood samples were collected from 6 affected individuals for chromosome karyotyping. The results showed that no numerical or structural abnormalities were found in these epileptic individuals. Furthermore, genomic DNA was isolated from whole blood cells for array-based comparative genomic hybridization (array-CGH) analysis. The data showed that duplication was identified in chromosome 14q11.1-11.2 from epileptic individuals of this family. The identified genomic gain regions were verified in all 6 affected individuals by real-time quantitative PCR and the results were consistent with those from array-CGH. These results indicated that chromosome 14q11.1-11.2 may be a candidate region for epilepsy and used for further investigation to determine the genetic factors of the familial epilepsy.

P1-4:

BRACHYTELEPHALANGIC CHONDRODYSPLASIA PUNCTATA. A RARE X-LINKED SKELETAL DYSPLASIA PRESENTING AS PIRIFORM APERTURE STENOSIS AND CAUSED BY A NOVEL MUTATION IN THE ARYLSULFATASE E (ARSE) GENE

Brian HY CHUNG[1,2], **David Chitayat**[2]

[1]*Department of Paediatrics & Adolescent Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong;* [2]*Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario*

Brachytelephalangic chondrodysplasia punctata (B-CDP) (OMIM 302950) is a relatively benign form of CDP. It was first described by Sheffield (*J Pediatr 1976; 89:916-923*) and subsequently discovered to have an X-linked mode of inheritance by Maroteaux (*Hum Genet 1989; 82:167-170*). In 1995, Franco et al (*Cell 1995; 81:15-25*) confirmed by positional cloning that the condition is caused by a defect in the ARSE gene. We report a patient with B-CDP presenting with respiratory distress and narrowed nasal passage. A novel mutation in the ARSE was detected. **Case report:** Our patient was born at 34 weeks gestation. His mother had gestational DM requiring insulin 2 weeks prior to delivery. Otherwise the pregnancy was uncomplicated and antenatal ultrasounds were normal. There was no maternal history of autoimmune diseases or exposure to teratogens. Delivery was spontaneous and vaginal and the APGAR scores were normal. His birth weight was 2360gm, body length was 44.2cm and head circumference was 32.5cm (all ~ 50th centile). He had high forehead, frontal bossing, hypoplasia of supraorbital ridges, depressed nasal bridge, malar hypoplasia, micrognathia, short fingers with broad and squared finger tips bilaterally and hypotonia. No other abnormalities were detected and there were neither cataract nor ichthyosis. Recurrent respiratory distress and desaturations after birth required repeated oral intubations since there were difficulties in passing the tube nasally. CT scan of the face showed right anterior piriform aperture stenosis. Additionally, the nasal cartilage was found to be deficient and there were stippling at the paranasal sinuses, nasal septum, posterior arch of C1 and odontoid. Skeletal survey confirmed the puncta at C1 and revealed additional puncta at the lumbar vertebrae, bases of the distal phalanges of fingers and toes, the calcaneus bilaterally as well as the left mid tarsal region. The distal phalanges of fingers and toes appeared shortened and broadened. The karyotype was 46,XY and maternal screening for lupus was negative.

DNA analysis of the ARSE gene (GeneDx) showed a hemizygous A>G substitution in exon 7, resulting in a replacement of threonine with alanine at position 306 (p.Thr306Ala). This has not been previously described but from multiple sequence comparison, this threonine codon is evolutionarily conserved across various orthologs and paralogs and is thus most probably disease causing. Functional analysis of this mutation is in progress. A recent review by Nino et al (*Am J Med Genet 2008; 146A:997-1008*) showed that so far 57 male patients with features of B-CDP have undergone ARSE mutation analysis, mutations have been detected in 31 but there were no common mutations to enable genotype-phenotype correlation. Respiratory problem is present in 30% of cases with ARSE deficiency. With the prominent nasal hypoplasia, obstructed nasal passage causing respiratory distress is expected to be common in patients with B-CDP and need to be addressed. High index of suspicion is important for making the diagnosis.

P1-5:

ANTI-PSYCHOPHARMACO RESULTED IN HIGHER RISKS OF SISTER CHROMATIC EXCHANGE OF SCHIZOPHRENIA AND MORBIDITY RATE OF TUMORIGENESIS FOR 16-YEAR FOLLOW-UP

Keqing Ding

Medical school of Yangtze University, Hubei Jingzhou, China

We used sister chromatic exchange (SCE) technology and tested 570 psychotic who was from 18-25 years in May 1988-Oct 1991. 280 of 570 were treated with chlorpromazine and 290 of was treated with Lithuanian carbonate. 330 normal people were as a control group. We compared the 3 group's SCE frequency before and after taken medicine, the interval of SCE testing is 2 months. The results shown: (i) that it was lower SCE frequency of control group than that of 2 psychotic before taken medicine, and there was the significant differences between control and patient groups ($p<0.01$). But there was no significant differences between 2 patient group before taken medicine ($p>0.05$), it implied that schizophrenia has certainly somewhat genetic substance damaged; (ii) we compared patient groups before and after taken medicine, we found that it had higher SCE frequency after taken medicine and there is statistical significant ($p<0.01$); (iii) the SCE frequency had very high differences ($p<0.01$) between the groups of Lithuania carbonate and chlorpromazine treatment. The results shown that morbidity rate of tumorigenesis in control group was 3.03% (1 of 330 had tumorigenesis) and 12.15% (69 of 570 had tumor) in the group of anti-psychotic drugs treatment. Conclusion: it demonstrated that anti-psychopharmacy may be potential mutant and the mutation is greater of chlorpromazine than that of Lithuania carbonate through 16 years follow-up investigation.

P1-6:

DIAGNOSIS OF INBORN ERRORS OF METABOLISM USING TANDEM MASS SPECTROMETRY AND GAS CHROMATOGRAPHY MASS SPECTROMETRY

HAN Lian-shu, YE Jun, QIU Wen-juan, GAO Xiao-lan, WANG Yu, GU Xue-fan

Shanghai Institute for Pediatric Research, Xinhua Hospital, Shanghai Jiaotong University School of Medical, Shanghai 200092, China

Objective: To improve the accuracy of diagnostic for inborn errors of metabolism, the tandem mass spectrometry was used to detect the amino acids and acylcarnitines in dry blood filter paper, the gas chromatography mass spectrometry was used to detect the organic acids profiles in uria. **Method:** The dry blood spots and the uria from a total of 5827 children suspected of inborn errors of metabolism were collected. The amino acids and acylcarnitines in the dry blood filter papers were tested by tandem mass spectrometry; the organic acids profiles in uria were tested by gas chromatography mass spectrometry. **Results:** In all of these patients, 362 cases (6.4%) were diagnosed. Among these disease, 173(47.8%) cases were diagnosed as amino acid diseases (113 hyperphenylalaninemia, 16 maple syrup urine disease, 13 ornithine transcarbamylase deficiency, 13 Citrullinemia type II, 5 Citrullinemia type I, 10 tyrosinemia type I, 2 homocystinuria, and 1 Arginasemia); 173(47.8%) cases were diagnosed as organic acidemia(90 methylmalonic acidemia, 20 propionic acidemia, 21 multiple CoA carboxylase deficiency, 15 glutaric acidemia Type I, 10 isovaleric acidemia, 7 β-keto thiolase deficiency, 5 3-methylcrotonylCoA carboxylase deficiency, 3 3-hydroxy-3-methylglutaryl-CoA lyase deficiency, 1 mevalonic aciduria, and 1 fumaric aciduria); 16 (4.4%)cases were diagnosed as fatty acid disorders(5 medium chain acyl-CoA dehydrogenase deficiency, 4 Very Long Chain Acyl CoA Dehydrogenase Deficiency, 3 short chain acyl-CoA dehydrogenase deficiency, 2 multiple acyl-CoA dehydrogenase deficiency, 1 carnitine palmitoyl transferase deficiency type I, and 1 carnitine palmitoyl transferase deficiency type II,). **Conclusion:** It is suggested that many inborn errors of metabolism can be diagnosed only with tandem mass spectrometry or gas chromatography mass spectrometry, but the diagnosis of a part of inborn errors of metabolism need the two methods.

P1-7:

A NEW ROLE FOR MATRIX EXTRACELLULAR PHOSPHOGLYCOPROTEIN /OSTEOBLAST FACTOR 45 IN DNA DAMAGE RESPONSE

Shuang Liu[1], Hongyan Wang[2], Xiang Wang[2], Lin Lu[2], Peter S.N. Rowe[3], Baocheng Hu[1,2]* and Ya Wang[2]*

[1]Beijing Institute of Biotechnology, Beijing 100850, China; [2]Department of Radiation Oncology, Kimmel Cancer Center of Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA; [3]Department of Internal Medicine, Kidney Institute & Division of Nephrology University of Kansas Medical Center, Kansas City, KS 66160, USA

Keeping balanced levels of proteins involved in DNA damage response is one of the essential steps for mammalian cells to reduce the risk of genomic instability. Here we show a new role for Matrix extracellular phosphoglycoprotein/osteoblast/ osteocyte factor 45 (MEPE/OF45) cloned in 2000 with the functions related to bone metabolism, in protecting cells from DNA damage-induced killing through maintaining CHK1 levels. By using PCRselect cDNA subtraction method from one pair of transformed rat embryo fibroblast cell lines that have similar genetic backgrounds but have different CHK1 levels and different sensitivity to DNA damage inducers, we identified MEPE/OF45 highly expressed in the resistant cell line. MEPE/OF45 interacts with CHK1, which prevents CHK1 from degradation and reduces the interaction of CHK1 with the ubiquitine E3 ligases (Cul1 and Cul4A). These data suggest a competitive mechanism for MEPE/OF45 to keep CHK1 from ubiquitine-mediated proteolysis. Our results demonstrate that besides the role in bone metabolism through extracellular secretion, MEPE/OF45 also plays an important role in DNA damage response through its maintaining CHK1 level in mammalian cells.

P1-8:

PREPARATION OF RECOMBINANT HEPARIN-BINDING DOMAIN POLYPEPTIDE OF FIBRONECTIN AND STUDY OF IN VIVO EFFECT OF THE POLYPEPTIDE ON SEPSIS IN MICE AND ON DISSEMINATED INTRAVASCULAR COAGULATION IN RATS

ZOU Qi-lian[1,2], CHEN Yuan-zhong*[1], CHEN Xiao-Fang[1], WU Yong[1], CHEN Ping[1], HUANG Mei-juan[1]

[1]Fujian Institute of Hematology,Union Hosptal, Fujian Medical University, Fuzhou 350001,China; [2]Division of Cell Biology and Genetics, Fujian Medical University, Fuzhou 350004

The gene of amino-terminal heparin-binding domains of the Fibronectin (403bp-1113bp), which codes for 237 amino acids (Ser46-Gly282), MW 29 kDa, was amplified from FNcDNA by high fidelity PCR. The aim gene cloned into pGEM-T vector, pAo815SM vector and then pPIC9K vector. It was expressed in Pichia pastoris cells. Polypeptide (rhFNHN-29) was purified by S-100 column and SP column thereafter. The polypeptide was proved able to bind with heparin but not with FN antybody by Western-blot. The experimental study of rhFNHN-29 polypeptide effect on mice with Sepsis and rats with DIC indicate that the rhFNHN-29 polypeptide has an effect on both sepsis in mouse model and DIC in rat model. It can reduce significantly the mortality of endotoxemia mice sensitized by GalN, saline control group 70%, polypeptide group only 15%. Less necrosis on the hepatocyte of polypeptide treated mice than on the saline one. The ultrastructure of hepatocyte under the electronmicroscope showed that cell apparatus of saline one were destroyed and cytoplasm became loose, polypeptide treated lightly. The TNFα level in the plasma of mice treated with saline was significantly higher than that of the rhFNHN-29 polypeptide treated. The expression level of TNFα, IL-1β, IL-6 mRNA on hepatocyte on the saline treated mouse was significantly higher than polypeptide treated. In the experiment of rhFNHN-29 polypeptide treated DIC rats model, the thrombosis in the lungs of the saline control was more severe and extensive than that of rhFNHN-29 polypeptide treated. The capillary hemorrhage in the live of the saline one was more severe and extensive than that of the rhFNHN-29 polypeptide treated. The plasma TNFα level in the saline one was higher than that of the rhFNHN-29 polypeptide treated significantly ($P<0.01$). The WBC, Hb, Plat and Fbg of the rhFNHN-29 polypeptide treated group were significantly higher than that of the saline

(P<0.01), PT and APTT significantly lower than that of the saline (P<0.01).

P1-9:

STS GENE IN A FAMILY WITH X-LINKED ICHTHYOSIS

Weiying Jiang, LiDu, Hongli Xi

Department of Medical Genetics Sun yat-sen University

X-linked ichthyosis (XLI) is X chromosome-linked recessive inherited disorder characterized by brown, adhesive, polygonal scales of skin. A lot of studies have indicated that XLI is relevant to the deficiency of steroid sulfatase (STS). More than eighty percent of XLI patients display large deletions involving in the entire STS gene, including the flanking regions on both sides, while a few point mutations and partial deletions have also been reported. So far, most STS gene deletions are identified by polymerase chain reaction（PCR）, southern blot, and fluorescence in situ hybridization (FISH). A few researchers identified carriers and made prenatal diagnosis for XLI family by FISH. However, this method is expensive, technically demanding, and may provide a false negative result in partial deletion XLI patients. There are three patients in the XLI family. The clinical presentation and the history of a similar skin condition in the proband's male maternal relatives helped establish the diagnosis of X-linked recessive ichthyosis. The exon1 and intron10 of STS gene from the XLI patients failed to amplify, but the inner control fragment was amplified successfully. Meanwhile, the normal members of the family and 2 unrelated normal subjects showed a normal amplification in these regions. These results revealed that there was a complete deletion of the STS gene from the XLI patients. Based on gene dosage effect, we firstly established a technique to detect ichthyosis carrier by DHPLC. Proband's mother and grandmother were identified as carriers, while proband's sister was a normal member. This technique lays a substantial foundation for gene diagnosis and prenatal diagnosis of XLI. We hope that this approach will be applied widely.

P1-10:

A NOVEL MUTATION IN A CHINESE ALAGILLE SYNDROME PATIENT WITH CUTANEOUS MANIFESTATIONS

Ling Xie, Rensheng Lai

Department of Pathology, Affiliated Hospital of Nanjing University of TCM

Alagille syndrome (AGS) is an autosomal dominant disorder characterized by five major symptoms: paucity of interlobular bile ducts, characteristic facies, posterior embryotoxon, vertebral defects and peripheral pulmonic stenosis. We report a Chinese boy who was finally diagnosed as a case of Alagille syndrome (AS) presenting with jaundice, pruritus and widespread papules, in which the diagnosis is suspected but the criteria for clinical diagnosis are not met, molecular genetic testing of *JAG1* was considered to confirms diagnosis of AS. The case is reported to highlight the fact that early recognition of the skin lesions and molecular genetic testing of *JAG1* may play a significant role in the diagnosis of this disease.

P1-11:

AN AUTOSOMAL DOMINANT HIGH MYOPIA LOCUS IN 12Q22 AND EXCLUSION OF *LUMICAN* AS THE CANDIDATE GENE

Tsz Kin Ng, Ching Yan Lam[1], Pancy O.S. Tam, Dorothy S.P. Fan, Coral W.S. Lee, Dennis S.C. Lam, and Chi Pui Pang

Department of Ophthalmology & Visual Sciences, The Chinese University of Hong Kong, Hong Kong; [1]Present affiliation: Department of Surgery, The Hong Kong University, Hong Kong

Purpose: Myopia is the most common eye disorder in the world. Heredity has been suggested to be an important contributor. There are 18 putative myopia loci mapped and 7 genes found to have association with myopia. In this study we explored the genetic component of a Chinese family in Hong Kong with autosomal dominant high myopia. **Methods:** Whole genome linkage analysis was performed on 13 members of the family. The chromosomal region identified from the initial scan was further analyzed by genotyping additional microsatellite markers. Candidate gene screening was performed by direct DNA sequencing. **Results:** From whole genome scan, a maximum two point lod score of 1.51 was observed at marker D12S351. Fine mapping revealed a maximum two point lod score of 2.71 at marker D12S88. A narrowed linkage region from 12q21.31 to 12q22 by haplotype analysis in the family. *Lumican (LUM),* which is located within this region, was screened and no segregation of polymorphism was observed within the pedigree. **Conclusions:** The mapped HM locus on chromosome 12 in this study confined a narrower linkage region of 4.42cM within the reported *MYP3* locus. *LUM* was excluded to be a candidate gene from this family.

P1-12:

FAMILIAL TRANSLOCATION t(10;18)(p15;p11.1): IDENTIFICATION AND FOLLOW-UP OF FIVE UNBALANCED OFFSPRINGS AND PRENATAL DIAGNOSIS OF THREE CASES

Yue-qiu Tan, Lu-yun Li, Ling-li Huang, Guang-xiu Lu

Institute of Reproduction & Stem Cell Engineering, Xiang-ya School of Medicine, Central South University, Changsha, Hunan 410078, China

We report on a familial translocation (10;18)(p15;p11.1), which transmitted in at least four generations. Five offsprings with unbalanced translocation were found in this pedigree. Two of them were partial monosomy 18p with partial trisomy 10p15→pter and three others were partial trisomy 18p with partial monosomy 10p15→pter. Compared with sporadic cases, these familial cases of unbalanced translocation of 18p and 10p more clearly showed that the phenotypes of the same structural abnormality involving 18p vary in familial individuals and further supports the fact that no special clinical syndrome is closely associated with the karyotype of 18p trisomy or monosomy. Through ten years of follow-up, we found the patients had no shortened life-span, and had simple self-care ability, and the intelligence could be improved through special education. Study of the family history is also useful to the success of prenatal diagnosis for three carriers with unbalanced translocation. One had a carrier baby, one had a normal baby, and another experienced three pregnancies, the former two of which were abnormal and had to be terminated, the third of which was healthy. No spontaneous abortion occurred in all carriers. The study indicated that some special segregation models of hereditary mechanism exist in the family.

P1-13:

CARNITINE-ACYLCANITINE TRANSLOCASE DEFICIENCY: CLINICAL AND MOLECULAR ASPECTS

B.Z. Yang, J.H. Ding, J.M. Mallory, R.J. Chai, and C.R. Roe

Institute of Metabolic Disease, Baylor Research Institute, Dallas, Texas, USA.

The carnitine-acylcarnitine translocase (CACT) deficiency, an autosomal recessive disorder, is one of the most severe defects of mitochondrial fatty acid oxidation, which presents as two clinical phenotypes: a severe neonatal onset form with cardiomyopathy and a milder phenotype with hypoketotic hypoglycemia. In this study, six patients with CACT deficiency, including both phenotypes were investigated. Incubation of patients' fibroblasts with [16-(2)H(3)]palmitic acid and analysis by tandem mass spectrometry revealed an increased concentration of [16-(2)H(3)]palmitoylcarnitine, suggesting the diagnoses of either CACT or Carnitine palmitoyltransferase II (CPT II) deficiency. CACT activity in fibroblast was almost absence and confirmed the diagnosis of CACT deficiency. To investigate the molecular basis of CACT deficiency, all of CACT exons, including intron and exon boundaries, were amplified and sequenced. RT-PCR was also performed to amplify CACT coding region. Sequencing of the patients' cDNA and genomic DNA for CACT revealed seven mutations in our group including an 8-bp deletion in CACT exon 1. In addition, in our total six unrelated patients with CACT deficiency, the correlation of mutant genotype to clinical phenotype was briefly discussed.

P1-14:

12 YEARS OF TRADITIONAL CHINESE MEDICINE GENETICS STUDIES

Tan Cong–e[1], Wang Mi--qu[1], Wang Hong-zhan[1], Han Yu-ping[2]

[1,2]Chengdu University of Traditional Chinese Medicine,TCM Genetics Research Center, (ChengDu 610075)

It has been 12 years since the first monograph of “Genetics of Traditional Chinese Medicine” was published and “the first international academic conference of Genetics of Traditional Chinese Medicine” was hold. As a newborn subject that combines the development of TCM with modern genetics, Genetics of TCM places its emphasis on the questions related to the functional genome in the fields of Chinese traditional medicine and Chinese materia medica. It is characterized on the theory of medical classics to study the information, network and rules of heredity and hereditary diseases, especially those polygenic inheritance diseases (in the modern medicine) or syndromes of multifactorial inheritance (in the TCM). Over the last 10 years, the Foundation of National Natural Science has provided financial support to a number of research topics of TCM which involved in genomics, functional genome, behavioral genetics, immunogenetics, molecular genetics, epigenetics, the history of genetic ideal, etc. That contributed a lot to the updating of theory; expand of the experiment and application of clinical care of TCM Genetics. The basic theory of TCM Genetics contains modern genetics and genetic thinking of the traditional Chinese medicine. The latter includes the Yin and Yang holism theory, the theory of kidney is congenital, Li Yi number theory, the theory of visceral manifestation, congenital endowment theory, etc. During the last 12 years, the research on TCM Genetics mainly shows in the following eight aspects: survey on family, twins of combining Western disease and the symptom of Chinese medicine, conduct genetic experiments of cat scaring rat utilizing natural enemies relations to make “fear injury kidney” model, immune gene (including TLR) expression profile and signaling pathway of deficiency of kidney yang, hypothalamus molecular biology mechanism of physiological aging mouse with kidney deficiency, the seven categories of energy metabolism with abnormal function in gene expression profile with cold pattern, promoting transcription and expression of γ-globin of β-thalassemia patients with Chinese medicine treatment, annoyance gene expression found in cerebral palsy children with deficiency of spleen and kidney, the immune and genetic observation by combination of Western disease and symptom of TCM on patients of diabetes, osteoarthritis. Finally this paper looks

forward to the future of TCM Genetics.

P1-15:

A PRIMARY STUDIES ON THE PLEIOTROPISM OF GENES AND THE EFFECTING QIHUA OF TCM GENETICS

Wang Hong-Zhan, Lin Qiao, Wang Mi-Qu

[1]Genetics Laboratory, Chengdu University of TCM(ChengDu 610075); [2]Visiting Research Fellow, Chengdu University of TCM

Through a lot of data analysis, there are conclusions as follows: For expression of a function subgenome-profile controlling disease are vary with the soma-constitution, age for inference in gene interaction and environment as climate, bacteria and virus etc, and inference to the vector of gene pleiotropism, therefore same gene can control different phenotype, symptom or disease at same body. And so on the process to form a functional profile in certain vector is similar with the conception as effecting theory in TCM of the Qihua (气化). Although any Qihua-effecting became pathological profile are vary at various stage of a disease or pathology but there are a symptom complex in general term exhibition on one or more of the Eight Pattern as Cold or Heat, Deficiency or Excess, Interior or Exterior and *Yin* or *Yang* and there are an effecting profile to control the phenotype responded respectively.

P1-16:

EXPLORING THE GENE PLEIOTROPISM AND THE QI-EVAPORATION FUNCTION IN TCM GENETICS

WANG Hong-Zhan, DUAN Azure Y. , WANG Mi-Qu, LIN Qiao

[1]Genetics Laboratory, Chengdu University of TCM (ChengDu 610075); [2]Visiting Research Fellow, Chengdu University of TCM

We have concluded from various publications that for a given sub-genome, the expression profile due to certain diseases is impacted by individual soma-constitution, age or the interaction among the genes, and environmental factors such as the climate, bacteria and virus. These factors also have inference on the direction of gene pleiotropism. Therefore the same genome can control different phenotype, symptom or diseases of the same body. At the same time, the process to form a functional profile in certain vector is similar to the conception of Qi-Evaporation in TCM. Although a pathological profile of Qi-Evaporation varies on different stages of a disease, one or more of the four TCM manifestations using the Eight Basic Pattern Theory could appear, with the Eight Pattern as Cold or Heat, Deficiency or Excess, Interior or Exterior and *Yin* or *Yang.* And each of the eight patterns has a corresponding Qi-Evaporation genome profile to control the phenotype of it.

P2-1:

EXPRESSION PATTERN OF *SMOOTHENED* IN MOUSE EMBRYONIC CRANIOFACIAL DEVELOPMENT

Juan Du[1], Zhipeng Fan[1], Xin Ma[2], Yan Wu[2], Shuhong Liu[2], Yan Shen[3], Ming Fan[2*], Songlin Wang[1,4*]

[1]Molecular Laboratory for Gene Therapy, Capital Medical University School of Stomatology, Tian Tan Xi Li No.4, Beijing 100050, P. R. China; [2]Institute of Basic Medical Sciences, Taiping Road No.27, Haidian District, Beijing 100850, P. R. China; [3]National Human Genome Center (Beijing) of China and National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing 100005, P. R. China; [4]Department of Biochemistry and Molecular Biology, Capital Medical University

Sonic hedgehog (*Shh*) signaling pathway plays many key roles in the development of *Drosophila* and vertebrate embryos including regulating craniofacial development. The seven-transmembrane protein smoothened (SMO) transduces the HH signal across the plasma membrane as an essential receptor of PATCHED1/2. There are few studies evaluating the particular expression of specific *Smo* involved in mouse embryonic craniofacial development. We investigated the expression patterns of *Smo* during murine embryonic craniofacial development using *in situ* hybridization (ISH) studies of whole-mount and sections, immunohistochemistry, quantitative real time PCR and Western blot analysis. We found that *Smo* was expressed at in the mouse embryo at 11 and 12.5 days postcoitum (dpc), after 13.5 dpc, the expression dropped to a low level and after birth, it was faintly detected; SMO protein could also be detected at 11, 12.5, and 14.5 dpc, after 15.5 dpc, the expression was very faint which confirmed the gene expression studies. Our findings on *Smo* expression provide a guide for further investigation of *Shh* signaling pathway gene function during craniofacial development.

P2-2:

THE IMPROVEMENT OF LMW RNAS PURIFICATION, THE CLONING AND EXPRESSION OF MIRNA IN HUMAN EMBRYO

Ying Wang, Su Yang

College of Life Science and Technology, Jinan University, Guangzhou, 510632, China

MicroRNAs (miRNAs) are the single strand small RNAs which are uncoding proteins. It is known that about 1/3 of Human genes are regulated by MicroRNAs. The unique cloning methods should be taken in miRNA researching because they are too small and have low abundance level. Such as termina enzyme designed by Pro. QU from Sun Yat-sen University in Guangzhou and the poly(A) tailing approach designed by Pro. Zheng from Medical College of PLA in China. The experiment improved the method of LMW RNAs purification which was based on the method of poly(A)-tailing. The concentration of the PEG and the precipitation time had been changed; the LMW RNAs were purified from the total RNAs successfully. Compared with the traditional methods, the novel method got more superiority .1. the LMW RNA from slight tissue or cell are purified; 2. it is more fast, simply and high efficacy; 3. PAGE electrophoresis is not necessarily used in order to lower the degradation probability of the RNA; 4. Isotope is not needed. MicroRNAs are valuable for the embryo development especially the early stage. The miRNAs had been found that are related to the embryo development and cell differentiation in c.elegan. 36 miRNAs had been discoved in embryonic stem cell of human. 8 small RNA sequences had been gotten from the embryo of 45days, 55days and 90days as well as the cord blood using the improved method successfully. After the BLAST and the second structure analysis, that one of the small RNA from material in the embryo before 90days was identified. This small RNAs might be miRNA, named miR-X1 temporarily. The miRNA sequence is CUACUCCUUCGGUCCAUG. Bioinformatics analysis showed that this miRNA was locate on intron of the forth chromosome p16.3 of human. By using half quota RT-PCR miR-X1 could express truly, so that miR-X1 was identified miRNA initially. But for further identify，there should be Northern blot Evidence. This miRNA had high expression in the embryo before 60days and cord blood, supposed that this miRNA might had the relationship with the cell differentiation and the development of human embryo. The result gave the few contributions to find the key miRNAs that control the development of human embryo as well as the cell differentiation.

P3-1:

STUDY THE POLYMORPHISMS OF HLA-A ALLELES IN PATIENTS WITH LEUKEMIA IN INNER MONGOLIA

CHEN A-mei[1], YUN Yu-guang , LIU Qing-ping[2*]

Department of Histology and Embryology, Inner Mongolia Medical College, Huhohote, .010059, Bioengineering College.DaLian University, Dalian 116622 [2]

Objective: HLA association has been considered as a possible genetic risk factor. To explore the correlation between the HLA-A alleles and susceptibility to leukemia in Inner Mongolia. **Methods:** HLA-A alleles polymorphism in patient of 87 were examined with flow cytometry-sequence specific oligonucleotide probes (FlOW-SSOP) methods. The normal subjects in Northern China as control. The allelic frequencies were calculated, and statistical differences between study groups were identified by use of the x^2 test. **Results:** Our data showed statistically significant differences between HLA-A in the control group and the investigated leukemia patient group. These results showed that The frequencies of allele HLA-A*11XX, HLA-A *31XX, HLA-A *6601were increased （$P < 0.05$）; while the frequencies of allele HLA-A*33XX were decreased in patients with Leukemia（$P < 0.05$）. **Conclusion:** HLA-A*11XX, A*31XX, A*6601 alleles may be positive correlated with Leukemia, while HLA-A*33XX, seem to contribute to its genetic resistance. These results suggested that positive association may exist between certain HLA genes and leukemias. These data suggest that host genetic background is an important factor in the etiology of leukemia. We hope that these results will be used as a guide for further functional studies.

P3-2:

ABERRANT MICRORNA EXPRESSION IN COLORECTAL CANCER

Xi Chen,[1] Xing Guo,[1] Hongjie Zhang,[1] Yang Xiang,[1] Guoqiang Wang,[1] Yi Ba,[2] Lingyun Zhu,[1] Kehui Wang,[1] Yuan Yin,[1] Xing Cai,[1] Jing Wang,[3] Rong Yang,[1] Yan Zhang,[1] Huajia Diao,[1] Jiangning Chen,[1] Ke Zen,[1] Junfeng Zhang,[1] and Chen-Yu Zhang[1]

[1]State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, China; and [2]Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Colorectal cancer is the third most common cancer and the second leading cause of cancer death in the United States. At the molecular level colorectal cancer arises from a series of genetic and epigenetic alterations that inactivate the tumor suppressor genes and activate the oncogenes. Recently, the classical family of protein-coding genes recognized as tumor suppressors and oncogenes has been expanded to include a type of non-protein-coding RNA molecules known as miRNAs. In present study we have screened the miRNA expression patterns in 6 pairs of matched colorectal cancer and normal adjacent tissue samples by both high-throughput real-time quantitative RT-PCR and miRNA microarray techniques. We show that a list of 73 miRNAs was significantly altered in colorectal cancer. Among these 73 miRNAs, a set of 15 miRNAs has been filtrated to act as an accurate biomarker in colorectal cancer diagnosis. In particular, among a panel of presumed targets generated by *in silico* analysis that may interact with key oncogenes or tumor suppressors, KRAS oncogene has been further experimentally validated as the target of miR-143. Our results imply that the loss of miR-143 may accelerate tumorigenesis via rescuing the suppression of endogenous miR-143 on KRAS. Taken together, the present study highlights a great potential of application of miRNA profiling in cancer diagnosis and provides the first evidences that miR-143 plays a significant role in cell growth inhibition and tumor suppression through inhibiting the KRAS translation.

P3-3:

HYPOMETHYLATION OF PREGNANCY-SPECIFIC BETA-1 GLYCOPROTEIN FAMILY LOCUS IN HUMAN GERM CELL TUMORS

Hoi-Hung Cheung[1,2], Tin-Lap Lee[1], Diana Taft[1], Owen Rennert[1], Wai-Yee Chan[1]

[1]Section on Developmental Genomics, Laboratory of Clinical Genomics, NICHD; [2]Department of Biochemistry, The Chinese University of Hong Kong

Human chromosome 19 is unique in that it has the highest gene density of all chromosomes. A number of gene families such as Krüppel-type zinc finger genes, olfactory receptors, and pregnancy-specific beta-1 glycoproteins (PSGs) are localized in this chromosome. Despite the high GC content of chromosome 19, the PSG cluster in 19q13.1-13.2, which harbors 10 PSG genes, has exceptionally low GC content. High level of PSG proteins was initially observed in placenta and in maternal circulation during normal pregnancy. They were though to function in the maintenance of pregnancy. However, PSG was also found in ectopic sites such as testis, intestine, and other non-placental tissues, suggesting these proteins may have non-pregnancy-related activities. In the present study, whole genome DNA methylation analysis by 5-methylcytosine immunoprecipitation combined with high resolution tiling array (MeDIP-chip) revealed a global hypomethylation in the PSG cluster in human germ cell carcinoma compared to non-tumor controls, indicating that hypomethylation of PSG genes might be associated with altered gene activity in male germ cell tumors. Examination of the relationship between DNA methylation of PSG genes and their transcriptional levels showed that hypomethylation of the PSG locus does not necessarily associate with increased expression of all PSG genes. Only the gene of specific species of the PSGs was up-regulated. Furthermore, methylation was not confined to the promoter but also in the intragenic and intergenic regions of the PSG locus. Treatment of 5-aza-deoxycytosine, a *de novo* methyltransferase inhibitor, reactivated the expression of some PSG genes. This suggests that the PSG genes are differentially expressed in tumors under epigenetic control. Most importantly, the PSG cluster lacks CpG islands, the hypermethylation of which in promoter regions is sometimes associated with gene silencing in cancers. We therefore hypothesize that non-CpG methylation may be important in the regulation of PSG genes.

P3-4:

RELATIONSHIP BETWEEN APOPTOSIS AND EXPRESSION OF P16, P130, NM23-H1 PROTEINS IN NASOPHARYNGEAL CARCIAOMA

Shengmiao Fu

The People's Hospital of Hainan Province, 19, Xiuhua, Xiuying, Haikou, Hainan, China. PR China 570311

Objective: To study the relationship between apoptosis and expression of p16, p130, nm23-H1 proteins or lymph node metastasis in nasopharyngeal carcinoma. **Methods:** Apoptosis was examined by TUNEL, p16, p130, nm23-H1 proteins were detected by S-P immunohistochemistry in 30 cases of nasopharyngeal carcinoma and 31 cases of chronic nasopharyngitis. **Results:** There is significant positive correlation between apoptosis and p16, p130, nm23-H1 positive expression or lymph node metastasis. The higher expression of p16, p130, nm23-H1 proteins is correlated with the higher AI, the lower lymph node metastasis and the better prognosis. **Conclusion:** The detection of apoptosis and the expression of p16, p130, nm23-H1 proteins has an important significance on pathogenesis of nasopharyngeal carcinoma and lymph node metastasis.

P3-5:

DIFFERENT EXPRESSION OF S100A8 AND S100A9 PROTEINS CONTRIBUTE TO THE DISSOLUTION OF COMPLEX S100A8/S100A9 IN LARYNGEAL CARCINOMA

Guo Yan, Liu Jia, Yang Qiang, Sun Kai-Lai and Fu Wei-Neng

Department of Medical Genetics, China Medical University, Shenyang, 110001, P.R.China

Calprotectin (S100A8/A9), a heterodimer of the two calcium-binding proteins S100A8 and S100A9, act as important pro-inflammatory mediator in chronic inflammation associated cancer. To explore the involvement and mechanism of S100A8/A9 complex in laryngeal squamous carcinomas, S100-interacted proteins were identified by immunoprecipitation together with mass spectrum. Co-localization of S100A8/A9 was obtained by immunofluorescence assay. The whole length of *S100A8* and *S100A9* genes were obtained from laryngeal carcinoma cell line Hep2 by PCR and DNA sequencing. Immunohistochemistry was used to detect the expression status and localization of both S100A8 and S100A9 proteins. As a result, immunoprecipitation and mass spectrum analysis revealed that 4 interacted proteins of S100A8 but not including S100A9 were detected. Immunofluorescence showed no overlapping fluorescence of S100A8 and S100A9 in Hep2 cell line. Sequence analysis revealed no abnormality within the nucleotides of *S100A8* and *S100A9* genes. Immunohistochemistry results displayed that S100A8 and S100A9, localized mainly in cytoplasm and nucleus, showed positive staining in well differentiated cancer tissues, meanwhile S100A8 also expressed in medium and poor differentiated tissues, which could explain why S100A8 doesn't combine S100A9 in Hep-2 cells. It concludes that S100A8 and S100A9 are not interaction proteins in medium and poor differentiated tissues of laryngeal carcinoma and the different expression of S100A8 and S100A9 proteins in different parts of cancer cells might be the radic for the dissolution of complex S100A8/A9.

P3-6:

HEIXUEDIAN, THE *DROSOPHILA* ORTHOLOG OF *TERE1/UBIAD1*, ENCODES A NOVEL MEMBRANE-BOUND PRENYLTRANSFERASE SUPPRESSING MALIGNANT BLOOD TUMOR

Ling Hong

College of Life Science and Technology, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan, P. R. China 430074

TERE1/UBIAD1 is a human disease gene involved in both multiple carcinoma and Schnyder crystalline corneal dystrophy (SCCD). The molecular mechanism for the involvement of *TERE1/UBIAD1* in both diseases is unknown.

We report the cloning of *heixuedian (heix)*, the *Drosophila* ortholog of *TERE1/UBIAD1*. Loss of function of *heix* in *Drosophila* results in uncontrolled division of prohematocytes, overproliferation of hemocytes, hypertrophy of the lymph gland and formation of melanotic tumors, similar to human lymphoma. In the hemolymph of *heix* larvae, the number of hemocytes increases about 50 times. The fraction of these hemocytes that are lamellocytes is increased, the morphology of these lamellocytes is altered, and they become competent to bind lectin, suggesting abnormal cell differentiation and inappropriate immunoactivation. The *heix* gene encodes a novel membrane-bound prenyltransferase. The HEIX protein is expressed in the embryonic hemocyte/macrophage precursors and in the larval lymph gland and hemocytes, as well as in the macrophage-like Schneider L2 cell line, suggesting a cell-autonomous role in tumor suppression. The subcellular localization of HEIX protein is on the cell membrane and in the apical side of cytoplasm. Our results provide the first direct evidence that *heix* is a bona fide tumor suppressor gene. Together with the human data, *heix/TERE1/UBIAD1* might bridge the lipid metabolism and tumorigenesis. Since *Drosophila heix* genetically interacts with hairless and hindsight, we propose that *heix/TERE1/UBIAD1* might use its prenyltransferase activity to control the proliferation and differentiation of *Drosophila* hemocyte possibly mediated by Ras and Notch signaling, respectively.

P3-7:

THE RESEARCH ON MICROSATELLITE INSTABILITY AND TUMOR

JING Qiang, WANG Jia, NIE Sheng-Jie, XIANG Chao-Jie, HU Li-ping, YIN Jian-Hua, YU Jian-Hua

[1]Department of Forensic Medicine, Kunming Medical College 650031, China; [2]Public Security Bureau in Kunming City, Kunming 650031*, China*

Objectives: In the research, we discuss the situation of microsatellite instability (MSI), loss of heterozygosis (LOH) and analysis the relationship between tumors mentioned above and chromogenes of human beings. We use traditional ammonical silver staining technique and 3100DNA automatic analyzer to compare the results. So as to discuss the reliability and effectiveness by using these two methods for tumor tissue in personal identity, identification in disputed paternity test, medical tangle and identification of the lost. **Methods:** Twenty cancer tissues taken from 20 different unrelated individuals and their blood specimens were examined with Chelex -100 and chloroform phenol extraction of DNA, then profile with PCR, we used polyacrylamide gel electrophoresis technique and 3100 Genetic Analyzer to analysis the amplification of the products. **Results:** The genetic alterations occurred in 8 out of 20 cases. Six STR loci mutate among 15 + 1 STR. MSI was most frequently observed at D5S818. **Conclusion:** This study seems to show the MSI plays a notable role in the occurrence of the bone tumor. The function of the 3100 Genetic Analyzer method is more stable, sensitive. Comparing to polyacrylamide gel electrophoresis technique the results are more accurate, clear and straight forward. The forensic community should take cautions to use the tumor tissue for personal identification.

P3-8:

THE EPITHELIAL GROWTH FACTOR RECEPTOR (EGFR) EXON DOUBLE-SEQUENCING ANALYSIS IN NSCLC

Ren-sheng Lai, Ling Xie, Long-shu Shen, Chang-le Zhu, Jun Qian

Department of Pathology, Affiliated Hospital of Nanjing TCM University, Nanjing 210029

Objectives: To study the mutation situation of Epithelial Growth Factor Receptor (EGFR) exon 18, 19 and 21 in Chinese non-small-cell Lung Cancer (NSCLC). **Methods:** Somatic mutation were identified for the forward and reverse sequence chains for the tyrosine kinase domain of the EGFR gene in 32 cases samples without Iressa-treated ,as compared with 10 volunteers blood control ,with preparing followed by the DNA template abstract quantified and PCR processing of Touchdown and purified. **Results:** We found nine mutations in 7 sample sequence of 32 non-small-cell lung carcinoma tissues, namely five reported mutation within exon 19, and two new heterozygous mutations that L833V and H835L within exon21, and two intron polymorphism .This results show mutation rate at 9/32 (28.13%) in Chinese with NSCLC, and at 31.58% in lung adenocarcinomas. **Conclusions:** EGFR mutation rate in Chinese with NSCLC is consistent with those of Asian women reported by 《Science》, but new mutation points in Chinese were presented as L833V and H835L.The mutation rate is concordance with domestic RR rate of NSCLC with Gefitinib in Chinese.

P3-9:

THE RESPONSIVENESS FOR INTRON 1 CA DINUCLEOTIDE REPEAT POLYMORPHISM AND MUTATIONS OF EPIDERMAL GROWTH FACTOR RECEPTOR TO MOLECULAR TARGETED DRUG TREATMENT OF LUNG CANCER

Jian-Rong Wang, Shu-Peng Zhang, Ren-Sheng Lai

Affiliated Hospital of Nanjing University of TCM, The Third Grade Laboratory of Molecular Biology of State Administration of TCM, Nanjing 210029, Jiangsu Province, China

Objective：CA repeat polymorphism in intron 1 $(CA)_n$ and EGFR mutations were analyzed for its association with molecular targeted drug responsiveness in lung cancer. **Methods**：Both observed groups consisted of 116 somatic specimens of lung cancer and control groups consisted of 20 peripheral blood samples were analyzed by direct DNA sequencing. Epidermal growth factor receptor mutations at exons 18, 19, 21 were analyzed from overall 136 specimens. CA repeat polymorphism in intron 1 of EGFR from 48 normal tissue or blood cell specimens was also analyzed by direct sequencing. 45 lung cancer patients were followed up for their treatment outcome of molecular targeted drug (EGFR Tyrosine kinase inhibitors) .In addition, we analyzed CA dinucleotide repeat polymorphism in intron 1 of EGFR followed EGFR mutations data in exons 18, 19, 21 for their association with clinical outcome in lung cancer patients treated with molecular targeted drug. **Results:** EGFR mutations were identified in 20 patients (17.25%) out of 116 somatic specimens which including 17 mutations in extron domain and 3 heterozygosis in intron domain. None mutation was found in 20 peripheral blood samples in control group. Although the mutant frequency in the adenocarcinoma (21.62%) was a little higher than in the squamous cell carcinoma（8.33%）and in other histological types（11.11%）of lung cancer, there was no significant difference between them. The rate of mutations in female patients (7/36, 19.45%) was a little higher than those in male patients (13/80, 16.25%), but there was also no significant difference between them. Response rate to EGFR TKIs was significantly higher in the patients with EGFR mutations (62.5%) than those without mutation (0%) (P < 0. 0l) in observed groups. Disease control rate to EGFR TKIs was significantly higher in the patients with EGFR mutations (100%) than those without mutation (44.4%) in observed groups (P<0. 05). In patients harboring EGFR mutations, disease control rate to patients treated with EGFR TKIs (100%) was

significantly higher than those who never treated with it (40%)（P＜0.05). Two of three patients with heterozygosis in intron domain treated with EGFR TKIs, the response rate was 0% and the disease control rate was 50%. The frequency distribution of EGFR intron 1$(CA)_n$ repeat in 48 cases was 23（47.9%）low CA repeat（CA≤16）and 25 (52.1%) high ones（CA＞16）in cases and controls. There was no statistical correlation between the length of CA repeat in intron 1 of EGFR and gene mutations, (P＞0.05,OR＜2). There was no significant difference in response rate and disease control rate of EGFR TKIs treatment between low and high both in patients with or without mutations（P＞0.05). **Conclusion:** Response rate (62.5%) (P < 0. 0l) and disease control rate (100%) (P<0. 05) to EGFR TKIs was significantly higher in the patients with EGFR mutations than without it. Our results once again show that somatic mutations of EGFR are a major determinant of EGFR TKIs response in lung cancer. There was no statistical correlation between the length of CA repeat in intron 1 and mutations of EGFR. There was no significant difference in response rate and disease control rate of EGFR TKIs treatment between low CA repeat patients and high repeat ones. As the presents of certain disease control rate in the non-mutation lung cancer patients treated with EGFR TKIs, the relationship between the length of CA repeat and molecular targeted drug sensitivity need to be studied furthermore under the solo factor.

P3-10:

ASSOCIATION ANALYSIS OF MUTATIONS OF CODON 1493 AND 1367 OF APC GENE IN MCR AND CLINICAL PATHOLOGY OF COLORECTAL NEOPLASM

FENG Li-fang[1], LAI Ren-sheng[2]

[1]Nanjing University of TCM, 210029, China; [2]Lab of Cytology & Molecular/Dept of Pathology, Affiliated Hospital of Nanjing University of TCM, 210029, China

Objectives: To evaluate the relationship of mutations in MCR (mutation cluster region) of exon 15 of APC (Adenomatous polyposis coli) gene and pathogenesis of colorectal. **Methods:** The genome DNA was extracted from 9 colorectal adenoma patients, 7 colorectal carcinoma patients and 27 healthy controls. MCR of APC gene exon 15 was amplified by PCR. The products were sequenced by gene sequencer. Then we analyze the mutations from the sequence we got. **Results:** Two types of APC gene mutations were identified as codon1493 (ACG > ACA), detected in 42 and codon1367 (CAG > TAG), detected in 1. Three kinds of nucleotide changes: G→A, G/A and C/T. The mutation rate of adenoma groups and carcinoma groups was 100%, control groups 96.3%. Most of nucleotide changes were G→A in the adenoma groups and control groups. G/A was the chief change in the carcinoma groups. There were no significant differences of any nucleotide change between each groups analyzed by Chi-square test. The comparison with 4478 base between G→A and G/A in total cases was shown the significant differences ($P<0.05$), also which got the significant differences in G→A with C/T ($P<0.05$) and G/A with C/T ($P<0.05$), respectively. **Conclusion:** Codon 1493 (ACG > ACA) mutation was synonymous mutation; there is no direct association between this mutation and colorectal neoplasm. Codon 1367 (CAG > TAG) mutation was nonsense mutation, which may be the oncogenic basic cause.

P3-11:

EXPRESSION OF ANTI-CYCLIND1 INTRABODY INHIBITS PROLIFERATION OF UTERINE CERVIX CANCER CELLS

Lihong Zhou[1], Xun Zhu[2], Yong Chen[1], Yuhua Cao[1], Desheng Zou[1], Guiying Li[1]

[1]Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130021, P. R. China; [2]Department of Immunology, School of Basic Medical Sciences, Jilin University, Changchun 130021, P. R. China

Objectives: The Objectives of this study was to inhibit the growth and proliferation of uterine cervix cancer cells through blocking and inhibiting the biological activity of cyclin D1 overexpressed in cancer cells by using intrabody technology. **Methods:** An expression vector pER-ADκ coding anti-cyclinD1 intrabody (ER-ADκ) was constructed by molecular cloning techniques and then was transfected into the uterine cervix cancer HeLa cells. The expression of *ER-ADκ* gene and antitumor effects of the gene products were observed by using RT-PCR, immunofluorescence, Dot blotting, growth curve, and FACS. **Results and conclusion:** *ER-ADκ* product in HeLa/pER-ADκcells can recognize and combine its target protein cyclinD1. The expression of ER-ADκ significantly inhibited the growth of HeLa cells. The antitumor rate was higher than 30%. The results of FACS showed that ER-ADκ arrested cell cycle of HeLa cells compared with the control, G0-G1 phase cells increasing by 20.23%, S phase cells decreasing by 11.41%, and increased the apoptosis of HeLa cells by 5.71%. ER-ADκ significantly inhibited the growth and proliferation of HeLa cells and distinctly induced apoptosis of HeLa cells. This study suggested that ER-ADκ might be further used in tumor gene therapy. [This study is supported by the Project for Young Scientists Funds of the National Natural Science Foundation of China (30200256) and the S&T Development Planning Program of Jilin Province (20050410-3)]

P3-12:

PC-1/PRLZ INTERACTS WITH AND REPRESSES ANDROGEN RECEPTOR TRANSCRIPTIONAL ACTIVITY

Hui Zhang, Jian Wang, Bo pang, Bangxiang Xie, Wu ruiqin, Xiaolong Qian, Lan Yu, Cuifen Huang, Jianguang Zhou

The Laboratory of Molecular Oncology, Beijing Institute of Biotechnology, Beijing 100850, P.R. China

Androgen receptor (AR) triggered by androgen action plays an important role in growth, differentiation and maintenance of prostate organs. In this study, we report a novel AR corepressor PC-1/PrLZ which has been isolated as a prostate cancer associated gene from prostate cancer cells. PC-1 is androgen induced and predominantly expressed in prostate tissue. Functional analyses conducted by the transient and stable transfection in prostate cancer cells revealed that PC-1 repressed AR transcriptional activity in androgen-dependent and androgen-independent prostate cancer cells. 2 Furthermore, a protein-protein interaction was identified between PC-1 and AR *in vitro* and *in vivo*. The N-terminal and leucine-zipper regions of PC-1 were responsible for the interaction with the DNA binding domain of AR. Electrophoretic mobility shift assay demonstrated that PC-1 repressed AR activity possibly through inhibiting AR binding to androgen responsible element (ARE). Moreover, the suppression of AR transactivation by PC-1 then resulted in altered patterns of gene expression of a series of androgen target genes including PSA, Nkx3.1 and NDRG1. Taken together, our findings have identified and characterized a novel AR corepressor PC-1, which may provide new insight into the molecular mechanisms mediating AR function and prostate cancer progression.

P3-13:

THE TRAIL GENE BY HTERT GENE CORE PROMOTER AND ITS EFFECT ON THE APOPTOSIS OF OVARIAN CANCER CELL LINE SKOV3

Li Hong-Mei, Song Tian-Bao, Yu Yue-Cheng, Xin Xiao-Yan, Zhana Ming, Wei Xiao-li, Xu Jing-Hong

[1]Department of Obstetrics and Gynaecology, Affiliated Hospital of Yanan University, Yanan 716000, China; [2]Department of Histology and Embryology, School of Medicine, Xian Jiaotong University, Xi'an, 710061 China; [3]Department of Obstetrics and Gynaecology, Xijing Hospital, Fourth military Medical University, Xi'an, 710032 China

AIM: To evaluate the effect of apoptosis of the TRAIL gene by the human telomerase reverse transcription gene core promoter on ovarian cancer cell line SKOV3 in vitro. **METHODS**: The TRAIL gene eukaryotic expression vector modulated by hTERT gene core promoter was transfected into human ovarian carcinoma SKOV3 cell line by lipofeclin mediation and positive clones were screened by G418, and the levels of mRNA were determined by reverse transcription polymerase chain reaction (RT-PCR). The cell apoptotic rate of SKOV3 was detected by flow cytometry. Apoptosis was detected by electron microscopy, DNA ladder and fluorescent staining. **RESULTS:** RT-PCR results showed that a 1049 bp fragment was amplified. The apoptotic rates of SKOV3-CMV-TRAIL and SKOV3-hTERT-TRAIL cells were 29.7% and 24.7%, respectively, higher than those of SKOV3 (12.6%) and SKOV3-CMV (12.9%) cells (P<0.01). Marked DNA ladder was observed by DNA gel electrophoresis, condensed chromatin beneath the nuclear membrane was detected by electron microscopy, and enhanced fluorescence and condensed or broken chromatin were shown by fluorescent staining, all of which indicated the apoptosis of transfected cells. **CONCLUSION**: The TRAIL gene by the human telomerase reverse transcription gene core promoter induced the apoptosis of the ovarian cancer cell line SKOV3 *in vitro*. The TRAIL gene eukaryotic expression vector modulated by hTERT gene core promoter had laid a solid foundation for further research about its role in regulating the biological behavior of ovarian cancer cells.

P3-14:

THE MECHANISM OF SPLUNC1 INVOLVED IN THE HOST DEFENCE OF NASOPHARYNGEAL EPITHELIUM

Xiaoling Li, Houde Zhou, Ming Zhou, Minghua Wu, Guiyuan Li

Cancer Research Institute, Central South University, Hunan 410078, P.R. China

We identified a tissue-specific gene, SPLUNC1, which was differentially expressed in nasopharyngeal epithelial and nasopharyngeal carcinoma (NPC) tissues，and has the bactericidal permeability-increasing protein/lipid-binding protein domain. We treated the pseudomonas aeruginosa by SPLUNC1 and the bacteria clones were counted, found that the SPLUNC1 inhibits the bacteria clone formation obviously. The LPS binding test also showed that SPLUNC1 can bind to LPS. We also observed the effects of SPLUNC1 and its poly-peptide on the EBV infected cells and found that both of the full length SPLUNC1 protein and its poly-peptide can promote the lysis and apoptosis of EBV infected cells, and the envelope of EBV was damaged and lost its integrality. Through green fluorescence protein (GFP) mediated subcellular localization experiments, we found a kind of tiny intracellular annular aggregates, approximately 50-400nm in size, in the NPC epithelial cell line HNE1, which could absorb the fluorescence of GFP-tagged SPLUNC1 protein. We verified that these microorganisms are nanobacteria (NB) with a negative staining using transmitted electronic microscope (TEM) and immunofluorescent analysis. From the biopsy specimen of NPC, we detected the NB, which can lead to the swelling of mitochondria in the infected host cells. These findings provide us a new relationship between antimicrobial and anti-tumorigenesis of SPLUNC1 and shed new light on the mechanism of SPLUNC1 involved in the host defense of nasopharyngeal epithelium.

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P3-15:

SIGNAL REGULATORY PROTEIN ALPHA1 IS INVOLVED IN THE INHIBITORY EFFECT OF GLUCOCORTICOID RECEPTOR ON MACROPHAGE RAW264.7 CELL PROLIFERATION

Xiao-hui Wang, Yi-dong Li, Yan Wang, Xiao-yan Zhu, Fei Diao, Jian Lu

Department of Pathophysiology, Second Military Medical University, Shanghai 200433, PR China

Glucocorticoid (GC) effectively suppresses immune and inflammatory responses and inhibits the growth of several types of cells, but the role of GC and its receptor on macrophage proliferation is unclear. In our previous work we found that the inhibition of glucocorticoid receptor (GR) expression in murine macrophage RAW264.7 cells by RNA interference resulted in significant enhancement of cell proliferation. In this study we found that the expression of signal regulatory protein alpha1 (SIRPα1) significantly decreased in RAW-GR (-) cells, and dexamethasone (Dex) could significantly increase the expression of SIRPα1 in RAW264.7 cells. Furthermore increased expression of SIRPα1 in RAW-GR (-) cells and RAW264.7 cells by transfection with SIRPα1 expression plasmid resulted in inhibition of cell proliferation, while inhibition of SIRPα1 expression by small interference RNA attenuated Dex-induced proliferation inhibition in RAW264.7 cells. In addition, we also demonstrated that increased proliferation was associated with significant elevation of expression of cell-cycle positive regulators (cdk2, cyclinD1 and cyclinB1) and increased activity of Akt, but not ERK1/2 and p38 in the RAW-GR (-) cells. Blocking the activity of Akt by using LY294002, a specific inhibitor of PI3K-Akt pathway, resulted in more obvious inhibition on growth of RAW-GR (-) cells than that of RAW-control cells. These results first demonstrated that induced expression of SIRPα1 by GC/GR was involved in the antiproliferative effect of GC/GR on RAW246.7 cells, and enhanced activity of Akt was also related to the increased proliferation of RAW-GR (-) cells.

P3-16:

PROTEOMIC ANALYSIS OF CERVICAL CANCER CELLS TREATED WITH ADENOVIRUS-MEDIATED MDA-7/IL-24

Lili Wei, Youquan Bu, Tao Cui, Songtao Ding, Faping Yi, Yongping Ma, Geli Liu, and Fangzhou Song

[1]Department of Biochemistry and Molecular Biology, Chongqing Medical University, Chongqing 400016, China

Mda-7/IL-24 is a novel member of IL-10 cytokine family, which has a significant anti-tumor activity without eliciting detrimental effects in normal cells. However, the molecular mechanisms underlying it were still elusive. In the present study, proteomic analysis was conducted to investigate the anti-tumor mechanisms by Ad.mda-7 in CaSki cervical cancer cells. A total of 43 differentially expressed proteins were visualized by 2-DE and silver stain., 29 proteins of which were identified via MALDI-TOF-MS analysis, 15 were upregulated (e.g. Tumor suppressor p53, Apoptosis regulator BAX, Adenylate kinase isoenzyme 1(AK1), Growth arrest and DNA-damage-inducible protein GADD45 gamma (GADD45γ)) and 14 were downregulated (e.g. Eukaryotic translation initiation factor 5A(eIF-5A), Protein DJ-1, Annexin V, Transcription elongation factor B polypeptide 2 (TCEB2), TRAF family member-associated NF-kappa-B activator (TRAF2), and c-Myc-responsive protein Rcl (RCL)). Among the identified proteins, the protein and mRNA alterations of six proteins were further confirmed by Western blot and semi-quantitative RT-PCR analysis. At both the mRNA and protein levels, p53, BAX, AK1, GADD45γ, and BCCIP were upregulated whereas eIF-5A was downregulated following Ad.mda-7 treatment. Taken together, our present findings may provide novel insights into the anti-tumor mechanisms by Ad.mda-7 in cervical carcinoma cells.

P3-17:

SILENCING OF POLO-LIKE KINASE (PLK) 1 VIA SIRNA CAUSES INHIBITION OF GROWTH AND INDUCTION OF APOPTOSIS IN HUMAN ESOPHAGEAL CANCER CELLS

Youquan Bu[1], Zhengmei Yang[2], Quanhai Li[3], and Fangzhou Song[1]

[1]Department of Biochemistry and Molecular Biology, Chongqing Medical University, Chongqing 400016, China [2]Laboratory Animal Center, Chongqing Medical University, Chongqing 400016, China and [3]Department of Immunology, Hebei Medical University, Shijiazhuang, Hebei Province 050017, China

Esophageal cancer ranks among one of the most frequent cause of cancer death in the world. Plk1 is overexpressed in human tumors and has prognostic value in many cancers including esophageal cancer, indicating its potential as a therapeutic target. In this study, we investigated the therapeutic potential of Plk1 in esophageal cancer using the technique of RNA silencing via small interfering RNA (siRNA). Synthetic siRNA duplexes against Plk1 were introduced into four esophageal cancer cell lines, which subsequently resulted in a significant inhibition in Plk1 expression in the cells. We found that the targeted depletion of Plk1 caused a dramatic mitotic catastrophe (mitotic cell cycle arrest, and defects in several mitotic events such as incomplete separation of sister chromatids and failure of cytokinesis) followed by massive apoptotic cell death, and eventually resulted in a significant decrease in growth and viability of all four esophageal cancer cell lines studied. In addition, our results also indicated that the mitotic arrest induced by Plk1 depletion is mediated by the inactivation of cdc2/cyclin B1 complex. Taken together, our study strongly suggested that Plk1 may serve as a potential therapeutic target in human esophageal cancer.

P3-18:

SURVIVIN GENE RNA INTERFRENCE INHIBITES PROLIFERATION, INDUCES APOPTOSIS AND ENHANCES RADIOSENSITIVITY IN CERVICAL CANCER CELLS

Hui SONG*, Xiao-Yan XIN, De-Tang WANG, Feng XIAO, Qiao-Hong YUE

Department of Obstetrics and Gynecology, Xijing Hospital, Fourth Military Medical University

Aim: Observed the effect of survivin gene RNA interference (RNAi) on proliferation, apoptosis, and radiosensitivity of human cervical cancer cell HeLa. **Methods:** Transfected the recombined eukaryotic expression plamid pSilencer2.1-s2 which concerned human survivin gene siRNA into human cervical carcinoma cell HeLa by using LipofectAMINE2000, selected the positive clones with G418, detected the expression of survivin mRNA and its protein by semi-quantitative RT-PCR and Western blot respectively, protracted cell growth curve by MTT assay, examined cell cycle distribution and cell apoptosis by flow cytometry, observed the changes of cell radiosensitivity by clonogenic survival assay. **Results:** Compared with other groups of HeLa cells, the expression level of survivin gene mRNA and protein declined evidently in the cells which having been transfected with pSilencer2.1-s2 plasmid, the expression inhibitory rate were 62.8% and 60.1%; the cell proliferation was inhibited, and the highest inhibitory rate was(57.8±2.1)%（P<0.05）; the changes of cell cycle distribution were obvious, many cells were blocked in G_0/G_1 phase(72.7±3.1)%（P<0.05）, G_2/M phase(5.1±2.9)% reduced sharply（P<0.05）; the apoptotic rate was (29.2±1.4)%, rising up obviously（P<0.05）; at the same dose of radiation, cloning efficiency declined notably（P<0.05）; the cell survival curve showed a significant decrease of D_0 and D_q too, were 3.15 and 1.21 respectively（P<0.05）; the radiation enhancement ratios were 2.01（a ratio of D_0）and 1.77（a ratio of D_q）. **Conclusion:** Survivin gene RNAi can inhibit proliferation, induce apoptosis and significantly enhance radiosensitivity through reducing the expression of survivin in human cervical carcinoma cells.

P3-19:

X-CHROMOSOMAL INACTIVATION SKEWING IS ASSOCIATED TO EARLY DEVELOPMENT OF ESOPHAGEAL CARCINOMA IN CHINESE FEMALES

Qin Su, Li Gong, Wei Zhang

Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences, Beijing; Department of Pathology, Tangdu Hospital, the Fourth Military Medical University, Xi'an, China

Skewed X-chromosome inactivation (SXCI) has been linked to development of ovarian, breast and pulmonary carcinomas, but its frequencies in healthy Chinese females and patients with esophageal carcinoma remains unknown. DNA was extracted from the peripheral blood cells from 401 Chinese females without a detectable tumor and 143 female patients with esophageal carcinoma. Exon 1 of androgen receptor gene was amplified, and its products of different alleles were resolved on denaturing polyacrylamide gels and visualized after silver staining. Corrected ratios (CRs) of the products before and after *Hpa*II digestion were calculated. When CR≥3 was used as a criterion, SXCI was found in two (4.3%) of the 46 neonates, 13 (7.8%) of the 166 younger adults (16-50 yrs) and 37 (25.7%) of the 144 elderly females (51-96 yrs), with the frequency higher in the elderly subjects than in the two former groups. When a more stringent criterion (CR≥10) was used, SXCI was found in one (2.2%), two (1.2%) and 16 (11.1%) of the subjects in the three age groups, respectively, its frequency being higher in the elderly than in the younger age groups. The overall frequencies of SXCI, as defined as CR≥3, in the patients and healthy female adults were 9.7% and 8.8%, respectively. However, the phenomenon was significantly more frequent in the patients aging ≤50 yrs (35.7%) compared to the corresponding reference group (7.8%). In the current study, SXCI was demonstrated in apparently healthy females from China, with its frequency associated to age. Its presence may be a predisposing factor for the early onset of esophageal carcinoma.

P3-20:

LRRC4 INHIBIT GLIOBLASTOMA CELL PROLIGERATION BY RTK SIGNALING PATHWAY

Minghua Wu, Enyi Liu, Zuping Zhang, Dan Li, Guiyuan Li

Cancer Research Institute, Central South University, Hunan 410078, P.R. China

Glioblastomas are the most common and lethal human primary brain tumors, and are characterized by high invasiveness, neoangiogenesis, and extended necrosis. The transformation of normal cells into gliomas occurs as a result of the stepwise accumulation of a series of genetic lesions. Leucine-rich repeats containing 4 (LRRC4) is a potential glioma suppressive gene. The conspicuous absence of LRRC4 in high-grade gliomas directly contributes to increasing tumor grade. The re-expression of *LRRC4* can significantly suppress glioblastomas U251 cells tumorigenesis *in vivo* and cell proliferation and invasion *in vitro.*LRRC4 expression in glioma cells significantly down-regulated K-RAS and phosphorylation of c-Raf, ERK, AKT, NF-kBp65, p70S6K and PKC, and up-regulated JNK2 and p-c-Jun, suggesting that LRRC4 inhibited receptor tyrosine kinase (RTK) signaling pathways. In addition, we also demonstrated LRRC4 inhibited U251 cells proliferation through the ERK/Akt signaling pathway by using inhibitor (PD98059 and LY294002) treatment, but not through the Akt-Raf-ERK signaling pathway. The data demonstrate that LRRC4 plays a major role in suppressing U251 cell proliferation and invasion by regulating the K-ras/Raf/ERK/Akt/NF-κBp65, p70S6K/PKC, and JNK2/c-Jun pathways. In a word, these results suggest a possible important ‘‘cross-talk’’ between LRRC4 and RTK-mediated intracellular pathways that can link signals of cell proliferation, chemotaxis and invasion in glioblastoma.LRRC4 may represent a new target for development of new therapeutic strategies in glioma.

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P3-21:

THE ANALYSIS OF HPV16 E6 VARIANTS AND HLA CLASS II POLYMORPHISM AMONG CERVICAL CANCERS

Yuping Wu, Benrong Liu

State Key Laboratory of Biocontrol, Department of Biochemistry, School of Life Sciences, Sun Yat-Sen (Zhongshan) University, Guangzhou, 510275, P.R. China

Infection with human papilloma virus type 16 (HPV16) confers a high risk for the development of cervical cancer. Variants of this virus may interact differentially with host genetic factors, possibly affecting the disease pathogenesis. This study was designed to investigate the association between HPV16 E6 variants and human leukocyte antigen (HLA) polymorphism within a Chinese population. Peripheral blood from HPV16 positive Chinese women with cervical carcinoma, who had previously been tested for HPV16 E6 variants, was used for HLA class II typing. In HPV16 positive cervical cancers, 23.6% of them were belonged to prototype, 65.5% were Asian variant, 5.5% were African type 1 and 3.6% were European variants. It was found that there was a significant positive association between DQB1*060101 allele and HPV16 As variant-positive cervical cancers (OR, 4.47; Pc=0.0018). A negative relationship was found between DRB1*150101-DQB1*0602 haplotype and decreased risk for HPV16 As variant-positive cervical cancers (OR=0.31; p= 0.037). Similar tendency was observed for the haplotype DRB1*070101- DQB1*0201 with HPV16 As variant-positive cervical cancers (OR=0.16, p=0.024). Additionally, as for the HPV16 E6 prototype-positive cervical cancers, a significant positive association was found in DQB1*060101 allele (OR=5.95; p=0.002; Pc=0.036), and similar trends were observed for DQB1*030201 (OR=10.87, p<0.0001; Pc=0.0018), and DPB1*1301 (OR=7.40, p=0.002; Pc=0.04). It was found that there was no significant association between DRB1-DQB1 haplotype and HPV16 prototype-positive cervical cancers. These data indicate that host genetic factors, such as HLA polymorphism, may determine the potential oncogenicity of the HPV16 E6 variant. The results suggest that a specific match between E6 variant proteins and HLA class II alleles may contribute to HPV16-related cervical carcinogenesis in a certain Chinese population.
Footnote：The research was supported by the National Natural Science Foundation of China (30772333 and 30160090).

P3-22:

THE CINSTRUCTION OF REGULATING EXPRESSION VECTOR AND TREATING VECTOR TARGETING THE AFP POSITIVE HEPATOMA

Xiaohua Yuan, Fuying Liu, Huanling Zhang, Long Zheng, Shuxia Song, Junxia Wang

Department of Molecular Biology, Hebei Key Lab. Hebei Medical University, Shijiazhuang 050017, PR China

Aim: To make the treatment of specific gene products only to anti-liver cancer cells without damaging normal cells of the liver and other normal cells; to improve the therapeutic effect; to avoid drug side effects of gene therapy we ligated the most remote AFP enhancer Ⅲ with the promoter to construct a 1.2 kb sequence of gene expression regulation and cloned it into pEGFP-N1 plasmid without CMV promoter. In AFP positive hepatoma cells we proved its relative specificity. Meanwhile, in order to validate these gene sequences' possibility in the treatment of the liver cancer. At the regulatory sequence 1.2 kb downstream we connected it with the tumor suppressor gene P53 and prove it relative specificity of the cell cycle arrest and apoptosis in different cells. **Methods:** 1. Construct of pAFP-EGFP plasmid. 2. Transfect pAFP-EGFP into HepG2, SMMC7721, HeLa cells, under the fluorescence microscopy to observe fluorescent protein expression. 3. Construct of pAFP-P53-EGFP plasmid. 4. Transfect pAFP- P53-EGFP into HepG2, SMMC7721, HeLa cells, the non-transfected HepG2, SMMC7721, HeLa cells as control groups.Detect the difference of p53 protein expression with Western blot method. 5. Transfect pAFP- P53-EGFP into HepG2, SMMC7721, HeLa cells. Examine the percent of G1 phase cells and the rate of apoptosis by Flow cytometry. **Results:** 1. The expression of green fluorescent protein in HepG2 cells was significantly higher than in the SMMC7721, HeLa cells by the drive of AFP promoter. 2. The P53 fusion protein expression is relative higher in AFP positive cells than in AFP negtive cells by the drive of AFP promoter. 3. The rate of apoptosis and the percent in G1 phase cells of HepG2 cells was higher than SMMC7721, HeLa cells. The %S of HepG2 cells was significantly lower than SMMC7721、HeLa cells.

P3-23:

CLINICAL FEATURES AND MISMATCH REPAIR GENES ANALYSES OF CHINESE SUSPECTED HEREDITARY NON-POLYPSIS COLORECTAL CANCER: A COST-EFFECTIVE SCREENING STRATEGY PROPOSAL

Hong-Li Yan, Li-Qiang Hao, Hei-Ying Jin, Qing-He Xing, Geng Xue, Qian Mei, Lin He, Shu-Han Sun

Institute of Genetics, Second Military Medical University, 800 XiangYin Road, Shanghai, P.R.C. 200433

China has the largest numbers of hereditary non-polyposis colorectal cancer (HNPCC) patients based on its population of 1.4 billion. However, the clinical data and mismatch repair (MMR) gene analyses have been limited. Here we performed microsatellite instability (MSI) and immunohistochemistry (IHC) analyses on a series of patients with a high-risk for HNPCC: 61 patients with family histories fulfilling Amsterdam criteria II (ACII-HNPCC) or suspected HNPCC criteria (S-HNPCC), and 106 early onset colorectal cancer (CRC) patients. Sixty late-onset CRC patients were used as control. Methylation of the *hMLH1* promoter was analyzed on tumors lacking hMLH1 expression. MMR germ-line mutations were screened on patients with tumors classified as MSI-H/L or negative for IHC. We identified 27 germ-line MMR variants in the 167 patients with a high-risk for HNPCC while only one germ-line mutation in hMSH6 was found in the late-onset CRC group. Of those, 23 were pathogenic mutations. The high incidence of gastric and hepatobiliary cancers coupled with the increasing number of small families in China reduces the sensitivity (43.5%, 30.4%) and positive predictive value (PPV) (45.5%, 17.9%) of the ACII- or S-HNPCC criteria. Considering that all 12 tumors with pathogenic mutations in *hMLH1* also showed promoter unmethylation, the sensitivity of IHC in conjunction with *hMLH1* promoter methylation analysis is not reduced, but the PPV was increased from 27.8% to 61.1%, and the total cost was greatly reduced.

P3-24:

EXPRESSION OF SMO, HIP AND PDGFRα IN ESOPHAGEAL CANCERS

Ling Yang, Shuhong Huang, Yuehong Bian, Xiaoli Ma, Hongwei Zhang

Institute of Developmental Biology, School of Life Sciences, Shandong University, Jinan, P.R. China

The hedgehog (Hh) signaling pathway plays an important role on cell growth and differentiation during normal embryonic development as well as in carcinogenesis. The over-expression of Hh and its target genes, *Gli1* and *PTCH1* in the tissues of esophageal primary tumors has been reported. SMO-antagonist, cyclopamine, or the sonic hedgehog neutralizing antibodies inhibited growth of esophageal cancer cells and induced apoptosis. However, expression of other members of hedgehog signaling pathway in esophageal tumors is not known. Here, we report expression of *Smo*, *Hip* and *PDGFRα* genes in the primary esophageal carcinomas. Expression of *Hip* transcript is correlated with expression of *Shh, PTCH1* and *Gli1*, suggesting that Hip is one of the target molecules of Hh signaling pathway in esophageal cancers. Expression of *PDGFRα* is restrictively expressed in squamous cell carcinomas. Our results indicate that activation of the hedgehog pathway occurs frequently in esophageal cancers.

P4-1:

PREDICTION OF B-CELL EPITOPES OF INTIMIN IN EHEC O157:H7 BY BIOINFORMATICS APPROACHES

Chengsong Wan, Yong Zhou

School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, 510515, China

Escherichia coli O157:H7 is associated with hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic-uremic syndrome in humans. Several bacterial components have been implicated in intimate adherence of EHEC to epithelial cells and to cause the attaching-effacing (A/E) lesion *in vivo*. Intimin, encoded by *eae* gene, is the most important protein for A/E lesion. Many studies discovered that the C-terminal 300 residue region of Intimin was the eukaryotic cell-binding domain. Recently, the C-terminal 300aa region of Intimin was explored as the candidate of antigen for vaccine on some experiments. In this study, the C-terminal amino acid sequence of Intimin was retrieved from the GenBank accession No.CAA77642 using the LASER GENE program of DNA STAR. The sequence information was used for the prediction analysis of antigenic determinants using PROTEAN programme of DNA STAR. In present study we predicted B-cell epitopes of EHEC O157:H7 intimin by six methods, such as prediction of secondary structure by Chou-Fasman, hydrophilicity by Hopp-Woods, flexibility by Karplus-Schulz, accessibility by Emini and antigenic index by Jameson-Wolf. It showed that B-cell epitopes were probably located at or adjacent to 76-88 aa region and 262-278 aa region, because their prediction values are high and present simultaneously on six parameters. The result will be validated by experimental studies. Our data are useful for immunoinfectomic studies and the development of better vaccines for EHEC O157:H7 infection.

P4-2:

IMMUNE GENE FUNCTION MODULE PREELECTED FROM KIDNEY-YANG DEFICIENCY PATTERN-GENOME

Wang Mi-qu, Han Yu-ping, Yang Hong-ya, Deng Xue-mei

Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China

Objective: This report outlines the latest development and achievements in genomes on kidney-yang deficiency pattern in China, and the immune gene function module preselected from Kidney-yang deficiency pattern is demonstrated. **Method**: A large-scale epidemic disease investigation was carried out, and typical patients with Kidney-yang deficiency pattern were chosen and analyzed from six aspects. **Results**: Results of microarray experiments demonstrated that the differentially expressed genes of Kidney-yang deficiency pattern, two classes of genes were most frequently appeared, e.g., immunological and metabolic genes. We thus proposed the first group of candidate immune genes for Kidney-yang deficiency pattern, among which 40 genes belong to metabolism. **Discussion**: It is a long-term project to investigate Kidney-yang deficiency pattern-genome, and we will face lots of difficulties and challenges. It is just beginning for the functional genes in Chinese Medicine, and the investigation on candidate genes is still at its initial stage with lower level, but it will be helpful to reveal the molecular mechanism of Kidney-yang deficiency pattern.

P4-3:

A GENOME-WIDE SCREEN FOR GENES AFFECTING LITHIUM SENSITIVITY IN *SACCHAROMYCES CEREVISIAE*

Jingwen Zhao, Xin Sun, Wei Lin, Xuehua Ma, Linghuo Jiang

Laboratory of Molecular Microbiology, Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, China

Lithium salts have been used in the treatment of depression and bipolar disorder over 50 years. The identification of an target of lithium in budding yeast as a model organism may help us to explain the molecular basis for the beneficial and toxic effects of lithium treatment. We describe here a genome-wide screen for genes affecting lithium sensitivity in *Saccharomyces cerevisiae*. After three consecutive screens, we found that deletion of each of 109 genes caused yeast cells sensitive to lithium and that deletion of each of 6 genes caused yeast cells resistant to lithium. Functional categorical analysis indicates that these genes are mainly involved in metabolism, protein fate and cellular transport.

P4-4:

PROTEOMICS AND RT-PCR ANALYSIS OF CARDIAC FIBROSIS

Jing Li, Xiang D. Tang

Department of Pharmacology, Nanakai University School of Medicine, 94 Weijin Rd, Nankai, Tianjin 300071, PR China

Heart failure remians a major mortality in developed countries even with the combinatory use of diuretics, β-blockers and angiotensin-converting enzyme (ACE) inhibitors. Cardiac fibrosis characterizes failing hearts, and limits cardiac repair and function in various heart diseases. Effort is being made to improve heart failure by intervening cardiac fibrosis but the progress is slow. We study cardiac fibrosis mechanisms from whole-animal, cellular and molecular levels in hope of finding new drug targets and we have established a cardiac fibrosis model by repeatedly injection of isoproteronol (ISO) (3 mg/kg/d x 5d) to Wistar rats. To further dissect how cardiac myocyte and fibroblast contribute to cardiofibrosis we employed RT-PCR and proteomic technique. Adult rat ventricular myocyte (ARVM) was islolated and cultured for 12 hr, followed by treatment with ISO (1 μM) for 24 hr. RT-PCR was used to analyze selected proinflammatory cytokines (MCP-1, MIP-1a, M-CSF), macrophage-activating molecules (GC-MAF, sialidase and galactosidase), collagen (I, III, IV), matrix metalloproteases (MMP2, 4, 7, 9) and TGF-β. Along with RT-PCR, we used 2D-PAGE analyze these molecules at protein level. Similar analysis was applied to cardiac fibroblast treated with ISO. Our study revealed novel results and provide new insight into the mechanisms underlying cardiac fibrosis.

P4-5:

TRANSCRIPTOMIC AND SYNTHETIC GENETIC ARRAY ANALYSIS OF YPR015C FUNCTION IN YEAST

JH Mao[1], M Shenwu[1], BB Kang[2], M Blue[1], Tanwir Habib[2], K Hill[1] and YP Deng[2]

[1]Department of Biology, Tougaloo College, 500 West County Line Road, Tougaloo. MS 39174, USA; [2]Department of Biological Sciences, University of Southern Mississippi, 118 College Drive, Hattiesburg, MS 39406, USA

The budding yeast *Saccharomyces cerevisiae* is a eukaryotic organism with extensive genetic redundancy. Large-scale gene deletion analysis has shown that over 80% of the ~6200 predicted genes are nonessential, implying that yeast cells can tolerate deletion of a substantial number of individual genes. In order to explore this functional redundancy, especially as it relates to transcriptional regulatory networks, an uncharacterized yeast C2H2 zinc finger proteins, YPR015C, has been chosen for this study. Little is understood about this protein. To better evaluate the function of YPR015C, we examined the gene expression patterns of the mutant strain *ypr015cΔ* using microarray analysis. The results indicate that a total of 185 genes differentially expressed with a cut-off $\rho < 0.05$ and fold change > 1.5 in *ypr015c*Δ. Functional categorization based on Munich Information Center for Protein Sequences (MIPS) revealed up-regulation of genes related to transcription and cell cycle, down-regulation of genes involving cell rescue and defense, suggesting a decreased response to stress conditions. Using high-throughput synthetic genetic array (SGA) analysis, we have identified 57 synthetic lethal interactions when query strain, *ypr015cΔ*, is crossed to an array of 4800 deletion mutants. Most of these genes that interact with YPR015c are involved in 6 different cellular processes including transcription and transcriptional regulation, glucose metabolism, fatty acid metabolism, response to stress, protein synthesis and uncharacterized functions.

P4-6:

ASSOCIATION OF POLYMORPHISM IN THE CHEMOKINE (C-C MOTIF) LIGAND 1 GENE AND 23 GENE AND PULMONARY TUBERCULOSIS IN A STUDY OF 1040 CHINESE PATIENTS AND 1020 POPULATION CONTROLS

Nelson Tang[1,2], Kathy Kong[1,2], KM Kam[3], CC Leung[3], CM Tam[3], J Blackwell[4], CY Chan[5]

[1,2]Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong; [3]Department of Health, Hong Kong SAR Government; [4]Cambridge Institute of Medical Research University of Cambridge, United Kingdom; [5]Department of Microbiology, The Chinese University of Hong Kong

The syntenic region of chemokines locus found in human chromosome 17q11.2 has been first linked to susceptibility to intracellular bacterial infection in mouse model. It was subsequently linked to predisposition to tuberculosis and leprosy in family study. The locus contains two clusters of CCL chemokine genes which are important candidate genes for susceptibility to intracellular pathogens. We carried out a large scale genetic association study of 1040 pulmonary tuberculosis patients and 1020 population controls. 30 Tagging SNPs of the CCL-cluster locus was defined to provide a comprehensive coverage of the haplotype variations in this locus in the Chinese population. Association was determined by single point chi-square analysis and correction for multiple testing. All genotype results followed HWE. Two tagging SNPs showed significant association with TB. The strongest signal was found in a SNP located in the genomic region of CCL1 ($p<1e-4$) which is on the gene CCL1. Another association signal at a SNP located at intronic region of chemokine gene CCL23. The frequencies of minor allele were 0.17 and 0.20 in the control and patients groups, respectively. Chi-square for genotype and allelic association showed p values of <1e-4. We are still investigating the functional property of CCL23 and CCL1. Both CCL23 and CCL1 are strongly induced by various pathogens. This cytokine is secreted by activated T cells and displays chemotactic activity for monocytes. Therefore, it is a putative key regulator of granulomatous inflammation in tuberculosis infection.

P4-7:

COMPILATION AND CHARACTERIZATION OF SNORNAS FROM FOUR REPRESENTATIVE ORGANISMS

Xie Jun

Fudan University, China

Accumulating in nucleolus as the largest population of small, stable RNA in eukaryotic cells, small nucleolar RNAs (snoRNAs) have been found to play important roles in RNA biogenesis. Until now the snoRNA data were available in distinct organism-specific databases. We compiled the wealth of data from four representative organisms into a single integrated resource featuring snoRNAs characteristics in sequences, and genome organizations, with the expectation that it servers as a useful information repertoire for the snoRNA study. The Online Sortable Database for Small Nucleolar RNAs (OSDCS) is a Web-based database of snoRNAs. Seven hundreds and sixty-seven entries, derived from a systematic literature curation and annotation effort, are currently maintained. The database is fully searchable and sortable through several search tools. With its use-case-oriented user interface, OSDCS allows researchers to browse and compare major features of known snoRNAs from four representative organisms. Our preliminary snoRNAs study based on the OSDCS survey indicates great diversities of snoRNA sequences and their genomic organizations in different organisms.

P4-8:

SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) ANALYSIS OF DIFFERENTIAL GENE EXPRESSION DURING PRE-IMPLANTATION AND IMPLANTATION

DING Yu-bin, He Jun-lin, Liu Xue-qing, Chen Xue-mei, Wang Ying-xiong*

Department of Genetics, Chongqing Medical University, Chongqing,China

The aim of this study was to profile the differential gene expression between peri-implantation time (day 3 of pregnancy, d3) and the implantation time (day 5 of pregnancy, d5) in pregnant mouse uterus by serial analysis of gene expression (SAGE). In our two SAGE libraries of 17-bp tags, the total numbers of tags sequenced were 16970 for peri-implantation uterus and 49880 for implantation uterus. There were 12220 tags expressed at d3, and 48420 tags expressed at day 5, of which 6250（51%）genes were single-matched on d3 and 20930（43%） genes on d5, respectively. Based on the ratio value between implantation period and pre-implantation period, there were 5970 tags significantly down-regulated (more than 2-fold) and 9380 tags significantly up-regulated (more than 2-fold) during implantation, or there were 277 tags significantly up-regulated (more than 5-fold) during d5 and 4450 tags significantly up-regulated (more than 5-fold) during d3. The tag ratio between d3 and d5 was more than 10-fold on 2750 significantly changed genes, and the tag ratio between d5 and d3 was more than 10-fold on 1100 significantly changed genes. The results demonstrate that many genes were involved in basic cellular activity, including catalytic activity, protein binding, protein biosynthesis, modification, catabolism, hydrolase activity, homeostasis, nutrition transport, signal transduction, metabolism, cell motility , cell migration, cell cycle, cell division, cell adhesion, cell growth, cytoskeleton organization and biogenesis, cell proliferation, cell differentiation, apoptosis, anti-apoptosis, regulation of transcription, immune defense response, antigen presentation, ribosome biogenesis, RNA processing, RNA binding, mRNA metabolism , DNA repair, DNA replication, development, and so on. Comparison of expression differences of specific gene(s) provides important information on the potential role of the particular gene at specific time points of the two critical stages. Our results also indicate that there were 17 tags which were significantly higher (more than 25-fold) expressed during d3 than during d5, including *Rps18* (ribosomal protein S18), *Rpl13* (ribosomal protein L13), *Cyp39a1* (cytochrome P450, family 39, subfamily a, polypeptide 1), *Spink3* (serine peptidase inhibitor, Kazal type 3), *Sub1*

(SUB1 homolog), *S100a6* (S100 calcium binding protein A6,calcyclin), *Tmsb10* (Thymosin, beta 10, mRNA), *Transcribed locus*(moderately similar to NP_705892.1 signal peptidase 21kDa subunit), *Serbp19* (Serpine1 mRNA binding protein 1), *Rpl27a* (ribosomal protein L27a), *Ciz1* (CDKN1A interacting zinc finger protein 1), *Itgb1* (Integrin beta 1, mRNA), *Cnn3* (Calponin 3, acidic, mRNA),*Tcp11* (t-complex protein 11), *Pdcd10* (programmed cell death 10), *4921517L17*Rik (RIKEN cDNA 4921517L17 gene), *Tsen34* (tRNA splicing endonuclease 34 homolog). These genes are responsible for metabolism, RNA binding, mRNA processing, protein biosynthesis, ribosome biogenesis, protein kinase activity, transcription, cell cycle, cell adhesion, cell proliferation, cell differentiation, apoptosis, regulation of cell migration, immune response, homeostasis, signal transduction and so on. We concluded that these genes maybe played a key role in the the formation of uterine receptivity during pre-implantation period.

P5-1:

MITOCHONDRIAL PHYLOGENOMIC STUDY OF 30 ORTHOPTERA SPECIES

Huang Yuan

College of Life Sciences, Shaanxi Normal University

The complete mitochondrial genomes of 25 species from Orthoptera were determined using primer walking with cloning sequencing strategy after Long PCR amplification. These mitochondrial genomes were comprehensively analyzed in properties with respects in gene content and rearrangement, base composition, codon usage, tRNAs and rRNA secondary structures, and structual domains in A+T-rich region. The most important conclusions listed as following: (1) *Ruspolia dubia* mitochondrial DNA contains a short A+T-rich region with 70bp in length. (2) An unusual feature of the *L. migratoria migratoria* mitochondrial genome is the presence of three tRNA-like structures. (3) The secondary structures of lsRNA and slRNA from 5 species were predicted and compared with those of other insects. (4) The conserved consensus structures of A+T-rich region were determined. (5) The gene order of *Erianthus versicolor* mitochondrial genome is same with that of in Ensefera species, and it have a relative higher evolution rate compared with other species. (6) The results phylogenetic relationships at families and superfamilies of Orthoptera reconstructed based on 30 complete mitochondrial genomes data confirmed a monophyletic Ensifera and Caelifera. Based on the comprehensive tree topologies, we can conclude that: 1) the monophyly of Tettigonioidea, Grylloidea and Gryllotalpoidea was confirmed, and Grylloidea has a close relationship with Gryllotalpoidea than Tettigonioidea; 2) the monophyly of Tettigoniidae was confirmed, and Tettigoniidae has a close relationship with Bradyporidae; 3) there is disagreement in the monophyly of Conocephalidae, and additional samples or other molecular markers are needed to get further proof; 4) the monophyly of Acridoidea was not supported; 5) Chrotogonidae and Pyrgomorphidae has a close relationship with Tridactyloidea than other Acridoidea insects; 6) the monophyly of Arcypteridae, Oedipodidae, Catantopidae and Acrididae was not be supported.

P5-2:

APPLICATION OF OLIGONUCLEOTIDE ACGH TO THE DIAGNOSIS OF MITOCHONDRIAL DNA DELETION AND DEPLETION SYNDROMES

A. Craig Chinault[1], Chad Shaw[1], Lin-Ya Tang[1], Lee-Jun C. Wong[1,*]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Background: Current method for the diagnosis of mitochondrial DNA (mtDNA) deletion syndrome is based on Southern analysis. The procedures are tedious and the results do not reveal the location of deletion or the percentage of deletion mutant heteroplasmy. The purpose of this study is to develop an oligonucleotide array-based comparative genomic hybridization (oligo aCGH) for the quick evaluation of mtDNA deletion, percentage of deletion heteroplasmy, breakpoints of deletion, and mtDNA copy number. **Methods:** A custom designed oligonucleotide based microarray was constructed to provide high density coverage of the nuclear genes involved in the biogenesis and the regulation and maintenance of mitochondrial function. The array also contains ~6400 probes covering both strands of the 16.6 kb mitochondrial genome. The performance of this array was characterized in more than 100 control samples and at least 30 samples with known mtDNA deletions. The oligo aCGH results were confirmed by sequencing to determine the DNA deletion breakpoints and by qPCR analysis to verify mtDNA copy number. **Results:** All samples with previously known deletions were clearly detected and the deletion breakpoints were correctly identified by the oligo aCGH. A previously unidentified deletion sample was also discovered. Extent of mtDNA depletion or percentage of deletion heteroplasmy in mitochondrial deletion cases can be estimated from the relative hybridization intensity values of the patient DNA and the age and tissue-matched reference DNA using a computational program we developed to implement the heteroplasmy and segmentation analysis. The power of this assay was particularly evident in two samples with confirmed molecular diagnosis of hepatic mtDNA depletion syndrome, one of which was shown to carry an intragenic deletion in the *DGUOK* gene. **Conclusions:** Our results demonstrate the strength of this custom oligonucleotide array for simultaneous detection of both mtDNA copy depletion and internal deletions, with elucidation of the deletion breakpoints and the percentage of deletion mutant heteroplasmy, as well as detection of intragenic nuclear gene deletions responsible for mtDNA depletion, if present.

P5-3:

NUCLEAR MODIFIER GENE *MTO2* MODULATES THE PHENOTYPIC EXPRESSION OF MITOCHONDRIAL DNA PAROMOMYCIN RESISTANCE MUTATION IN YEAST *SACCHAROMYCES CEREVISIAE*

Qingfeng Yan[1,2], Xinjian Wanga[2], Ge`rard Faye[3], and Min-Xin Guan[2]*

[1]College of Life Science, Zhejiang University, Hangzhou 310058, Zhejiang, P.R. China; [2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA; [3]Institut Curie, Section de Biologie, Centre Universitaire, 91405 Orsay Ce`dax, France

Nuclear modifier gene(s) have been shown to modulate the phenotypic expression of mitochondrial DNA mutations. We report here the identification and characterization of the yeast nuclear modifier gene *MTO2* encoding an evolutionarily conserved protein involved in mitochondrial tRNA modification. Interestingly, *mto2* null mutants expressed a respiratory-deficient phenotype when coexisting with the C1409G mutation of mitochondrial 15 S rRNA at the very conservative site for human deafness-associated 12 S rRNA A1491G and C1409T mutations. Furthermore, the overall rate of mitochondrial translation was markedly reduced in a yeast *mto2* strain in the wild type mitochondrial background, whereas mitochondrial protein synthesis was almost abolished in a yeast *mto2* strain carrying the C1409G allele. The amounts of mitochondrial $tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Gln}$, $tRNA^{Leu}$, $tRNA^{Gly}$ and $tRNA^{Met}$ were markedly decreased but those of $tRNA^{Arg}$ and $tRNA^{His}$ were not affected in mto2 strains. The *mto2* strains exhibited significant reduction in the aminoacylation of $tRNA^{Lys}$, $tRNA^{Leu}$ but almost no effect in those of $tRNA^{His}$. Interestingly, the strain carrying the C1049G allele exhibited an impairment of aminoacylation of those tRNAs. Furthermore, the steady-state levels of mitochondrial mRNA *CYTB, COX1, COX2, COX3,* and *ATP6* were markedly decreased in *mto2* strains. These data strongly indicate that unmodified tRNA caused by the deletion of *MTO2* caused the instability of mitochondrial tRNAs and mRNAs and impairment of aminoacylation of tRNAs. In addition, we showed that yeast and human Mto2p localize in mitochondria. The isolated human *MTO2* cDNA can partially restore the respiratory-deficient phenotype of yeast *mto2* cells carrying the C1409G mutation. These functional conservations imply that human *MTO2* may act as a modifier gene, modulating the phenotypic expression of the deafness-associated A1491G or C1409T mutation in mitochondrial 12 S rRNA.

P6-1:

DESIGN AND EVALUATION OF CONDITIONALLY ACTIVE GENE THERAPY VECTORS

D. R. Wood*, J. H. Ding*, B. Z Yang*, R. J. Chai*, N. Akilla*, P.A. Grayburn**

**Institute of Metabolic Disease, Baylor Research Institute, Dallas TX 75226, USA; **Baylor Heart and Vascular Institute, Baylor University Medical Center, Dallas TX 75246 USA*

Gene Therapy is an area of research that holds tremendous potential for the treatment of disease and restoration of health. We have previously demonstrated that a noninvasive method, ultrasound targeted microbubble destruction (UTMD) can be used to deliver genes *in vivo.* Briefly, intravenous microbubbles carrying VEGF gene were selectively destroyed within the myocardial microcirculation by low-frequency ultrasound and promoted angiogenesis. In this study, we designed and tested gene therapy vectors that are significantly up-regulated only under specific physiological/pathological conditions. Regulation of gene expression via hypoxia would be an ideal genetic switch for gene therapy vectors targeting hypoxic related diseases, such as ischemic heart disease. We utilized a modified cardiac alpha myosin heavy chain (MYH6) promoter to drive the reporter genes Luciferase and DsRed. The MYH6 promoter was modified in a number of variations, such as by the removal of the YinYang1 (YY1) motif sequences and by the addition of a Hypoxia Responsive Element (HRE) and Activator Protein-1 (AP1) repeat sequences. More than 50 vectors were constructed over the course of this project. All constructs were sequenced to confirm the orientation of the insert and the accuracy of the sequences. To achieve a quantitative value *in vitro* for each of our vectors, Qiagen Effectene transfection reagent was used and cells, including H9c2, L6 and fibroblast were grown under conditions of both normoxia (20% O_2) and hypoxia (1% O_2). Data was analyzed with Statview software (SAS, Cary, NC, USA). Our *in vitro* study results indicate that significant gene expression is possible via a hypoxia reactive promoter, and it may achieve both tissue specificity and hypoxia reactivity within a single promoter construct. Our constructs and study are a step towards this ultimate goal for gene therapy.

P7-1:

MICROSURGICAL ENUCLEATION OF TRIPRONUCLEAR HUMAN ZYGOTES AND A NEW HUMAN EMBRYONIC STEM CELL LINES WITH TRIPLOID KARYOTYPES

JIANG Yong-hua, SUN Xiao-fang*, YIN Yi-fei, LONG Xiao-lin, DU Hong-zi, CHEN Xin-jie, ZHENG Yu-hong, LIU Wei-qiang, XIAO Guo-hong

Institute of Gynecology and Obstetrics, The Third Affiliated Hospital of Guangzhou Medical College, Guangzhou, 510150, China

Background: Human oocytes fertilized *in vitro* which containing 3 pronuclei were discarded in clinic, but there is a report of live birth occurring after microsurgical correction of tripronuclear zygotes. In this study, we attempts to correction triploid zygotes to the diploid state by remove one male pronucleus, and establish diploid human stem cell from tripronuclear zygotes. **Methods:**14 tripronuclear zygotes were removal of one male pronucleus, cytochalasin B was used to assist pronucleus removal, morphological changes were observed in during extended culture.10 tripronuclear zygotes were cultured in sequential media until develop into blastocysts, the inner cell mass (ICM) were isolated by immunosurgery and seeded on mitomycin C-treated mouse embryonic fibroblast (MEF) feeder layers, hESC colonies were subsequently characterized by cell surface marker staining, karyotyping and teratoma formation. **Results:** 13 of 14(92.8%) tripronuclear zygotes survived after micromanipulation, 10 of them development into 2-cell stage, 6 developed into 4-cell stage, 4 developed into 8-cell stage, no developed into morula. 4 of 10 tripronuclear zygote developed into blastocysts and the ICM were isolated, 3 ES-like clones have been found, one new hESC lines were established.The new cell line karyotyping is 46, XXX, express specific cellular and molecular markers characteristic of undifferentiated hESC lines, remaining the potential to form all three embryonic germ layers *in vitro* and in vivo. **Conclusions:** Tripronuclear zygotes have the potential of developing into normal diploid state by microsurgical enucleation one pronucleus, but we do not get blastocysts after microsurgical treatment in this research. The hESC lines with chromosomal aberration may be used as a model for *in vitro* studies.

P7-2:

PROTEOMIC CHARACTERIZATION OF DIFFERENTIATION OF MOUSE F9 EMBRYONIC CARCINOMA CELLS INDUCED BY RETINOIC ACID

Rui-qing Jiao, Jen-fu Chiu*

The State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

Retinoic acid (RA) is a member of the most biologically active forms of retinoid. In tissue culture, RA induces cell differentiation and suppresses cell growth in a wide spectrum of cell lines. RA has been successfully used for the chemoprevention and chemotherapy of various types of cancer because it inhibits proliferation and induces the differentiation of many types of malignant cells. The multipotent mouse F9 embryonic carcinoma cell is an ideal model system to investigate the mechanism of RA in cell differentiation and cell growth control and the biochemical basis of early embryonic development.

We reported here a proteomics approach to study protein expression changes during the differentiation of F9 cells into the visceral endoderm. F9 cells were incubated with RA at 0, 24, 48 and 72 hours. Total proteins extracted were separated by two-dimensional electrophoresis (2DE) and the protein patterns on the gels were comparatively analyzed by computer. 16 proteins spots were identified by MALDI-TOF MS or ESI-MS/MS. These proteins included metabolism enzymes, HSP60s, RAN, hnRNP K, FUBP1, VDAC1, STI1 and prohibitin. These proteins are involved in cellar metabolism, gene expression regulation, stress response and apoptosis respectively. This is the first report to show that the expression of PURH, VDAC 1 and STI1 were altered dramatically in cells treated with RA. The data from proteomic analyze is consistent with the result obtained from Western blot analysis. This is the first study using comparative proteomic analysis to investigate the protein profile of F9 cells during differentiation induced by RA.

P7-3:

IMMORTALIZATION OF HUMAN HEMATOPOIETIC PROGENITOR CELLS HAVING BETA-THALASSEMIA MAJOR GENOTYPE

Yvonne K Kwok[1], Wen Deng[2], Mary HY Tang[3], Sai Wah Tsao[2], P. C. Ho[1], Elizabeth T. Lau[3]

[1]Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong SAR, China; [2]Department of Anatomy, The University of Hong Kong, Hong Kong SAR, China; [3]Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, Hong Kong SAR, China

We aim to immortalize hematopoietic progenitor cells (HPC) from the blood of a beta thalassemia major fetus at 16^{+2} week gestation. The affected fetus had two mutations in the β-globin gene: IVS II 654 splice mutation in one allele and δβ gene deletion in the other allele. CD34+ cells were isolated from the affected fetal blood, cultured for 6 days and stored in liquid nitrogen. Thawed cells were then transduced with a retroviral vector harboring the human telomerase catalytic subunit (hTERT). Human papillomavirus type 16 (HPV16) E6/ E7 oncogene was transduced into the cells 4 days later. Immortalization of another cell line was established by transducing first with HPV E6/E7 and then with hTERT 6 days later. Results from both cell lines showed that transduced cells proliferated continuously beyond 30 population doublings (~ one year) with a myeloerythroid progenitor phenotype ($CD33^{+}CD34^{-}CD38^{-}CD45^{+}CD71^{+}CD133^{-}$) while cultured in a serum-free condition in the presence of stem cells factor (SCF), Flt-3 ligand, thrombopoietin (TPO) and interlekin-6 (IL-6). Untransduced HPCs senesced at 11 population doublings. Analytical studies of transduced cells at 7^{th} and 22^{nd} passages confirmed the expression of HPV16 E6/E7, the presence of the mutations, and the absence of β-globin protein expression. Level of telomerase activity and karyotyping will further be performed. This represents the first reported human beta-thalassemia hematopoietic progenitor cell line and will be a useful *in vitro* model for further studies of β-thalassemia gene therapy.

P7-4:

DERIVATION AND CHARACTERIZATION OF HUMAN EMBRYONIC STEM CELL LINES FROM POOR QUALITY EMBRYOS

Weiqiang Liu, Xiaofang Sun*, Xiaolin Long, Yumei Luo, Yifei Yin, Yonghua Jiang, Wenhong Zhang, Hongzi Du, Shaoying Li, Yuhong Zheng, Qing Li, Xinjie Chen, Baoping Liao, Guohong Xiao and Weihua Wang

Institute of Gynecology and Obstetrics, The Third Affiliated Hospital of Guangzhou Medical College, Duobao Road, Guangzhou, 510150, People's Republic of China

This study was designed to investigate an effective method for the derivation of human embryonic stem cells (hESC) from poor quality embryos discarded from in vitro fertilization (IVF) laboratories. Poor quality embryos were donated from IVF centers on day 3, cultured in a blastocyst medium for 2 days, and then in a blastocyst optimum culture medium for additional 2 days. The isolated inner cell masses (ICMs) were inoculated onto the feeder layer of mouse embryonic fibroblasts for subcultivation. The biological characterizations and differentiation capability of these cells were examined after at least 15 passages. Thirty-two embryos developed to expanded blastocysts or hatched blastocysts and ICMs were successfully isolated from all embryos. Four new hESC lines were established after further culture. All hESC lines were positive for stage-specific embryonic antigen (SSEA)-4, tumor-rejection antigen (TRA)-1-81, TRA-1-60 markers and negative for SSEA-1 marker. They showed a high level of alkaline phosphatase activity, positive expression of transcription factor octamer binding protein 4 mRNA. Furthermore, these cells had the potential to form both embryoid bodies in vitro and teratomas in vivo. Three hESC lines had normal karyotypes and one had trisomy 13. In this paper we describe a stable and effective system for hESC isolation and established four new hESC lines, which will provide an excellent experimental model for cell therapy, developmental biology and genetic research.

P7-5:

EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS IMPROVE COGNITIVE FUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Jianfeng Lu, Houyan Song

Department of Molecular Genetics & the Key Laboratory of Molecular Medicine, Ministry of Education, Fudan University, Shanghai, P.R. China

Stem cells are self-renewing, pluripotent cells that can be manipulated in vitro to differentiate into virtually any cell type. Stem cells are highly proliferative and have the potential to expand into very large numbers of a desired cell lineage. As such, they represent an excellent source of cells for cellular replacement strategies in disease states that are typified by a loss of a particular cell population. In the present study, we attempted to explore cell transplantation therapy for Alzheimer's disease (AD) using embryonic stem cell-derived neural stem cells (ES-NSCs). Mouse embryonic stem cells were differentiated into nestin-positive ES-NSCs in vitro by alltrans retinoic acid (ATRA). ES-NSCs were transplanted into the lateral ventricle of an AD mouse model via stereotaxic injection and displayed extensive and positional incorporation in the brains of model animals with improved cognitive score assessed by the Morris water maze. Our results demonstrated that intraventricular transplantation of ES-NSCs might be an effective delivery system for neuronal lineage-committed progenitor cells moving toward the pathological sites of AD, and these data also suggested the potential neuroreplacement therapy of ES-NSCs for AD.

P7-6:

LONG-TERM CULTURE OF MOUSE SPERMATOGONIAL STEM CELLS UNDER SERUM-FREE CONDITIONS

Sun Yuan, Tao Ke, Fan Li-qing

Institute of Reproductive & Stem Cell Engineering, Central South University, Changsha, 410078

Introduction: Spermatogonial Stem Cells (SSCs) are the only germline stem cells in adults; they self-renew and produce a large number of differentiating germ cells. The improvements of SSCs researches not only provide a good platform for the study of spermatogenesis in vitro and self-renewal of stem cells, but also have important implications for the development of biological experimentation, medical research and transgenic technology. Thus, the aim of the present study was to establish a long-term proliferation culture system for mouse SSCs. **Materials/methods:** Testis tissues were obtained from newborn male ICR mice on postnatal day 2 to 6. Testis cell suspensions were collected by two-step enzymatic digestion prior to culture. The serum-free culture medium for the testis cells was StemPro-34SFM supplemented with StemPro supplement, GDNF, LIF, EGF, bFGF and other nutritional supplements. From the second passage, the cells were maintained on mitomycin C-inactivated mouse embryonic fibroblasts (MEF) feeders. BrdU incorporation test was used to determine its proliferation and, Alkaline phosphatase (AP) activity, immunofluorescence staining and RT-PCR assay were carried for the identification of the cultured cells. **Results:** The cultured SSCs remained in a steady state and continued to generate germ cell colonies. The undifferentiated state was confirmed by the strong positive for AP activity, immunofluorescence staining of GFRα-1^{+}/Oct-4^{+}/VASA^{+}/SCP3^{-} and GFRα-1^{+}/Oct-4^{+}/SCP3^{-} at the gene expression levels. **Conclusion:** SSCs could be expanded in our defined culture system and passaged steadily in vitro. The harvested cells remained in an undifferentiated state, which provides a good platform for the further study of their pluripotent potential.

P7-7:

DIRECTED DIFFERENTIATION OF PARTHENOGENETIC HUMAN EMBRYONIC STEM CELLS (HESCS) TO NEURAL PROGENITOR CELLS AND DOPAMINERGIC NEURONS

Ding Yuan, Pingyuan Xie, Ge Lin, Guangxiu Lu

*Institute of Reproductive & Stem Cell Engineering, Central South University, National Engineering & Research Center o*f Human Stem Cells, Xiangya Road 88#, Changsha, Hunan, China 410078

HESCs could provide available tools for neural development research and potential sources for cell replacement therapy. Parthenogenetic hESCs were more promising for less ethic issues, the differentiation ability of which remained unknown. We had established a parthenogenetic hESC line - chHES32 and more normal hESC lines, and our previous study showed that human feeder cells could well maintain the undifferentiation of hESCs. Here we performed neural differentiation either directly on human feeder cells or through the formation of floating EBs. After 7~9 days, cells in both groups took on the appearance of “rosettes”, and later turned into neural tube-like structures 5~7 days late. Cells in both groups had similar gene expression profiles with neural development related genes up-regulated and pluripotent genes down-regulated. We concluded that direct induction seemed to have a higher efficiency. Parthenogenetic and normal hESC-derived “rosettes” and neural tube-like structures were further induced into midbrain dopaminergic neurons by adding FGF8 and SHH into the differentiating medium. Both groups could differentiated into β-tubulin+ neurons and tyrosine hydrozylase+ cells, while cells derived from day 10 had a higher expression of midbrain dopaminergic neuron marker En1 and Nkx6.1. Thus the progenitors derived from “rosettes” could be more suitable for differentiation into midbrain dopaminergic neurons, and parthenogenetic hESCs had similar potentials of differentiating into neural progenitors and dopaminergic neurons. We considered the direct differentiation performed on human feeder cells more approachable for further transplantation therapy and parthenogenetic hESCs could be induced into neural progenitors and dopaminergic neurons as well.

P7-8:

T CELL CLONE DIRECTING AUTOGENEIC CELLS WAS FOUND IN PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

Yuan Ou, Ping Zhu

Department of Hematology, Peking University First Hospital 100034 China

Objective: Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for hematological malignant tumors, and allo-HSCT is widely used in patients. Patients'immune system may be dysfunctional because of pre-administration. Reconstitution of bone marrow contains both hemotopoietic and immune reconstitution, and the immune recovery needs longer time which is intimately associated with long-term survival of patients. T cell reconstitution is an essential factor in immune recovery, so it's important to identify the clonal expansion and distribution, quantity and function of different T cell clones in patients after HSCT. This investigation is aimed to determine the function of specific T cell clone in patients after HSCT, and discuss if this T cell clone is related to marrow failure. **Methods:** The patient was diagnosed as acute myelocytic leukemia (AML) M2 type (FAB criteria), and didn't acquire complete remission after chemotherapy for six months. So this patient had to achieve allo-HSCT when there were quantity of leukemic cells in vivo. The donor's HLA*A0201 was mis-matched with the patient, which can be looked as the marker of donor's cells grafted in the patient. In addition, due to NPM1 gene mutation in the patient, we can estimate if leukemia recurred by real-time quantitative PCR. Hematopoiesis of the patient had recovered for a short time and the patient experienced graft-versus-host disease (GVHD) at the second month and the sixth month after HSCT. At the fifth month after HSCT, the patient had a relapse of leukemia but relieved through comprehensive therapy, and after twelve months the patient experienced bone marrow failure. Erythrocytes, granulocytes and platelets decreased consecutively. Finally, the patient died as a result of the failure of the second transplantation and serious infection. We collected peripheral blood or bone marrow consecutively from the patient at different stages during 360 days after HSCT, and monitored TCR spectratypes to understand the characteristics of dominant T cell clone. Bone marrow chimerism analysis reflected the development of lymphocytes grafted into the patient and the TCR repertoire distribution could be obtained by TCR family specific antibody dyeing and flow cytometry. Furthermore, we separated the dominant T cell clone using TCR family specific antibody MiniMacs Column and

analyzed it's function through fluorometric assessment of T lymphocyte antigen specific lysis (FATAL). Meanwhile, we determined the dominant TCRαfamily by TCRAV repertoire analysis. After obtained dominant TCRαand TCRβfamilies, we amplified TCRαand TCRβchains which contained complete length from leading peptide to stop codon using switching mechanism at 5'end of the RNA transcript (SMART) and PCR. After constructing correct pVITRO2 expression vectors we transferred the target TCR gene into common T lymphocytes and Jurkat cells by nucleofection and lipofection. Also, the function of the engineered T lymphocytes was assessed by FATAL method. **Results:** We observed that TCRBV13.1 family began to expand in 60-70 days after HSCT, and the band on TCRBV spectratyping was intense in 120-165 days. Thereafter, TCRBV13.1 family was dominant continuously. The TCRBV13.1 band from TCRBV spectratyping at the 120 day were removed from the gels for direct sequencing and the result represented the T cell clone which expressed TCRBV13.1 family was monoclonal. Moreover, flow cytometry results beginning with 200 days after HSCT showed that TCRBV13.1 T cell clone was dominant not only in peripheral blood but also in pleural fluid continuously.The CD4/CD8 ratio of TCRBV13.1 lymphocyte clone was about 1:3-4.During the period after HSCT, the result of marrow chimerism analysis showed the patient's cells were all derived from the donor according to the HLA type changing from HLA-A*0210 and HLA-A*2402 into HLA-A*0201 and HLA-A*2402. Likewise, we got three dominant monoclonal TCRαfamilies: TCRA1, TCRA10 and TCRA24 by analyzing TCRAV spectratyping at the same period with TCRBV spectratyping. TCRBV13.1 T cell separation and function assessment results showed this clone could direct donor's peripheral cells, and the lysis rate was 11%.Then we amplified complete length of TCRA1,TCRA10,TCRA24 and TCRB13.1 gene and constructed four expression vectors: pVITRO2-EcoRV-TCRA1-SalI, pVITRO2-EcoRV-TCRA10-SalI, pVITRO2-EcoRV-TCRA24-SalI, and pVITRO2-EcoRV-TCRB13.1-SalI respectively. The results of TCR gene co-transfection showed that pVITRO2-EcoRV-TCRB13.1-SalI combined with pVITRO2-EcoRV-TCRA24-SalI had the most high expression rate on cell surfaces. So we ligated TCRB13.1 and TCRA24 into two different multiple cloning sites in the same pVITRO2 plasmid respectively. The TCRB13.1 was ligated between EcoRV and SalI and TCRA24 was between SgrAI and NheI. Finally, we transferred the vector through lipofection into Jurkat cells and assessed the function of engineered T cells using FATAL method. We observed TCRB13.1 and TCRA24 engineered T cells could direct donor's peripheral cells and the lysis rate was 43.16%.Interestingly,the engineered T cells could also recognize this patient's tumor cells and the lysis rate was 14.99%. **Conclusion:** After allo-HSCT, a T cell clone derived from the donor directing autogeneic cells was found in the

patient due to the different hematopoietic and immune conditions. Common T lymphocytes can be engineered to acquire antigen specificity by TCR gene transfer, which provided a new approach for immune therapy of tumors.

P8-1:

DETECTION OF THE S252W MUTATION IN FIBROBLAST GROWTH FACTOR RECEPTOR 2 (FGFR2) IN FETAL DNA FROM MATERNAL PLASMA IN A PREGNANCY AFFECTED BY APERT SYNDROME

PKC AU[1,3], YKY KWOK[2], KY LEUNG[1], MHY TANG[1], ET LAU[1]

[1]Prenatal Diagnostic & Counselling Department, Tsan Yuk Hospital, HK; [2]Department of Obstetrics & Gynaecology, The University of Hong Kong, HK; [3]Department of Medicine, The University of Hong Kong, HK

Apert syndrome is a congenital disorder characterized by craniosynostosis, midface dysplasia and syndactyly of the hands and feet. It is known to be inherited in an autosomal dominant fashion, but most cases are sporadic. The prevalence at birth is about 15 per million. Two mutations, S252W and P253R in Fibroblast Growth Factor Receptor 2 (FGFR2) account for most cases of Apert syndrome studied to date. Prenatal diagnosis of Apert syndrome has been reported by using two- and three-dimensional ultrasound; and magnetic resonance imaging in second or third trimester. Confirmation of the diagnosis is done by DNA sequencing or restriction enzyme digestion of fetal samples. We developed a novel method by combining restriction enzyme digestion and molecular beacons in qPCR to detect the S252W mutation in fetal DNA from maternal plasma. Results show that our designed molecular beacon probe can detect the point mutation when Dpn II is used to cleave the normal allele from maternal plasma in a pregnancy with an affected fetus at 32 weeks gestation. Maternal plasma from normal pregnancies are negative for the mutation. More affected samples would help to verify this non-invasive prenatal diagnosis of the S252W mutation in Apert syndrome.

P8-2:

TWO COMPOUND HETEROZYHOUS FETUS WITH LDL-R GENE MUTATION AFTER PRENATAL DIAGNOSIS

Jie Lin[1], Luya Wang[1], Shu Liu[1], Shangzhi Huang[2], Juntao Liu[3]

[1]Department of Arthrosclerosis, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China; [2]Department of Genetics, Chinese Academy of Medical Sciences and Peking Union Medical College , Beijing 100005, China; [3]Department of Gynaecology and Obstetrics, Peking Union Medical College Hospital, 100005, China

Introduction: As one of the major pathogenic factors of coronary heart disease, familial hypercholesterolemia (FH) becomes a hotspot in the field of lipid metabolism and coronary heart disease research. The pathogenesis of FH is due to the low-density lipoprotein receptor (LDL-R), apolipoprotein B-100 and proprotein convertase subtilisin/kexin type 9 gene mutation of which restrains the combination of LDL-R with low density lipoprotein (LDL), and therefore results in the increase of plasma cholesterol and the deposition of cholesterol in the tissues. Clinical studies demonstrated that serum levels of LDL cholesterol directly related arterial calcification, which predicted a heightened risk of myocardial infarction and sudden coronary death. **Objective:** To detect the mutation of LDL-R gene in Chinese fetus with familial hypercholesterolemia. **Methods:** The fetus' heart and the major large vessels were detected by vessel ultrasound examination and their LDL-R gene coding region was sequenced. **Results:** The novel heterozygous 1439C→T and 1729T→G mutations of LDL-R gene were detected in fetus 1. Echocardiogram showed the calcification of aortic valve and its root. Blood vessel ultrasound examination showed the crude of abdominal aorta intima. Two other compound heterozygous mutations of LDL-R gene 611G→A and 675delA were detected in fetus 2. **Conclusion:** The LDL-R gene mutation of the FH fetus led to the aortic valve calcification, which was the major phenotype. These mutations of LDL-R gene were firstly determined in the world. These data increase the mutational spectrum of FH in China. **Acknowledgements**: This work was partly supported by National Natural Sciences Foundation（30470722）， Beijing Natural Sciences Foundation（7052021, 7062010）and Science and Technology New Star Funds of Beijing（2004B27, 2005A29）

P8-3:

SINGLETON BIRTH AFTER PREIMPLANTATION GENETIC DIAGNOSIS FOR HUNTINGTON'S DISEASE USING WHOLE GENOME AMPLIFICATION

Chow JFC[1], Lau EYL[1], Ng EHY[1], Leung KY[2], Tong T[3], Lam S[3], Yeung WSB[1], Ho PC[1]

[1]Department of Obstetrics and Gynaecology, University of Hong Kong; [2]Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital; [3]Cytogenetic Laboratory, Clinical Genetic Service, Department of Health, HKSAR, Hong Kong

Objective: To provide preimplantation genetic diagnosis (PGD) for Huntington's disease using whole genome amplification. **Patients:** The husband (age 33) with the family history of Huntington's disease carries the expanded allele while the wife (age 32) is normal. **Intervention:** Fifteen oocytes were retrieved after ovarian stimulation and 11 oocytes were fertilized after intracytoplasmic sperm injection. On Day 3 of culture, one blastomere was biopsied from each of the 10 good quality embryos. PGD was performed on the biopsied blastomere using whole genome amplification followed by polymerase chain reaction (PCR) with fluorescence primers. Three pairs of primers were used for the amplification of *IT15* gene at the (1) trinucleotide expansion site; (2) trinucleotide expansion site plus the polymorphic site situated on its 3'-end, and (3) polymorphic markers located downstream of trinucleotide repeats. **Results:** Five embryos were diagnosed to be normal, 2 were abnormal and 3 with inconclusive results. Two normal blastocysts were replaced on day 5. Two good quality blastocysts were cryopreserved. Those embryos with inconclusive diagnosis were later confirmed to be abnormal. The wife gave birth to a normal girl around 34 weeks of gestation, whose genetic status was confirmed by aminocentesis. **Conclusion:** This is the first report on PGD for Huntington's disease using whole genome amplification. Whole genome amplification provides a universal platform for amplification of DNA from single blastomere. The method facilitates the downstream PCR procedures and avoids the optimization of tedious multiplex PCR. Furthermore, the method produces sufficient DNA material for the detection of an additional polymorphic marker to discriminate the embryo DNA from that of the laboratory staff as a quality control measure of the procedure.

P8-4:

PRENATAL GENE DIAGNOSIS OF MUCOPOLYSACCHARIDOSIS AND OSTEOCHONDRODYSPLASIS

Yibin GUO[1], Jingxin PAN [2], Chunmiao GUO[2], Yanfang ZHONG[3], Yaxian MENG[1], Jingjing WANG[1], Luming CHEN[1], Weiying JIANG[1], Chuanshu DU[1]

[1]Department of Medical Genetics, SUN Yat-sen Medical College, SUN Yat-sen University, Guangzhou 510080,China; [2]Department of Internal Medicine, The Second Affiliated Hospital, Fujian University of Medical Science, Quanzhou 362000, China; [3]Prenatal Diagnosis Center, Guangdong Women and Children's Hospital and Health Institute, Guangzhou 510010,China

The author is major in "gene diagnosis and prenatal gene diagnosis of genetic disorders". Since 2005, we have used some methods, such as microcrystalline DNA extraction, PCR, restriction enzyme analysis, DHPLC, DNA direct sequencing and direct identification of specific primer amplification, etc. using these methods; we have made gene diagnosis for both Mucopolysaccharidosis and Osteochondrodysplasia to the suspected patients and their parents. Meanwhile, on the basis of etiopathogenisis elucidation, we also started prenatal gene diagnosis to pregnant women who wanted to breed the second fetus. It is to be mentioned that these suspects are from more than 20 provinces: GuangDong (HongKong and Macao included), GuangXi, HaiNan, FuJian, HuNan, HuBei, SiChuan, YunNan, JiangShu, JiangXi, ShangHai, TianJin and ChongQing, etc. On Oct.10th, 2006, we diagnosed the pregnant women who were suscepted by B-mode supersonic wave as ACH fetus's mother. She came from GuangDong province and was at a gestational age of 23 weeks. The results notified that: c.1138G→A heterozygous missense mutation (p.G380R) in FGFR3 gene was found in the fetus, so the fetus was diagnosed as ACH patient and then was induced abortion. On May 10th, 2007, we detected IDS gene to the pregnant women (GuangDong province), who was at a gestational age of 20 weeks and had ever bred a Hunter syndrome patient. The results showed that the male fetus did not have gene mutation which came from proband, and other sites of IDS gene had not mutation yet. According to follow-up investigation, the phenotype of this male fetus is normal, and this is also totally the same with the result of prenatal gene diagnosis. On July 27th, 2007, we diagnosed another pregnant women (GuangDong province), the second suspect by B-mode supersonic wave as ACH fetus's mother, who was at a gestational age of 24 weeks. As a result, c.1118A→G heterozygous missense mutation

（p.Y373C） in FGFR3 gene was found in the fetus. Therefore, he was diagnosed as TD1 patient and was induced abortion. On Oct.27th, 2007, we diagnosed the third pregnant women, suspected by B-mode supersonic wave as ACH patient's mother, who was at a gestational age of 26 weeks. She also came from GuangDong province. The results notified that: one c.746C→G heterozygous missense mutation (p.S249C) in FGFR3 gene was found in the fetus. so he was diagnosed as TD1 patient and was induced abortion. On Jan. 22nd, 2008, we diagnosed the pregnant women of SiChuan province, who had a Morquio syndrome baby, and had ever made another Morquio syndrome fetus induced abortion. This time she was at a gestational age of 19 weeks. The results showed that there were heterozygous mutations in the coding regions both Exon 1and Exon 10 of GALNS gene in the fetus. They were compound heterozygote, and were the same with the proband's mutation type. So he was diagnosed as Morquio syndrome fetus and was induced abortion .On Mar.4th, 2008, we diagnosed the pregnant women (GuangDong province), who had two babies suffered from Hunter syndrome, and her three brothers died of the disease. She was at a gestational age of 20 weeks. The result notified that the female fetus was normal about Exon 9 of IDS gene at the family's common mutation site. Other Exons and Exon-Intron sites haven't been found abnormality, so it was permitted to continue pregnancy. According to the results of prenatal gene diagnosis in these years, we have successfully established steady prenatal gene diagnosis about two types of MPSII、MPSIV and ACH, TD1. The adopted methods include microcrystalline DNA extraction, direct identification of specific primer amplification have quick, specific and accurate effects, and we could obtain conclusions during 1-2 days. Even combined with DNA Sequencing, they only need 7 days, which are especially fit for quick prenatal gene diagnosis of high risk gravida.

P8-5:

NON-INVASIVE PRENATAL DIAGNOSING FETAL ANEUPLOID USING PRINS

Xu Xiao-Yan

Department of Gynecology and Obstetrics, Tongji Hospital, Tongji Medical college, Huazhong University of Science and Technology, Wuhan, China 430030

Obstective: To prenatal diagnosis fetal aneuploidies using multiple primed in situ labeling (PRINS) and investigate the possibility of a new credible technique in non-invasive prenatal diagnosis. **Method:** Flow cytometry was used to isolate the fetal erythroblast from 120 cases of maternal peripheral blood, and then PRINS was applied to detect the X, Y and 21 chromosomes in single fetal cells. **Result:** X chromosome was detected in every sample. The sensitivity and specificity were both 100%. Y chromosomes were detected in 69 cases. The sensitivity and specificity were 92% and 100%, respectively. In addition, 1 case of XXY syndrome and 2 cases of Down's syndrome were detected. **Conclusion:** PRINS is a reliable new technique for the gene analysis of fetal single

P8-6:

RETROSPECTIVE ANALYSIS OF ANTENATAL SCREENING OF 8341 CASES FOR DOWN'S SYNDROME

HU Yu, GAO Lan, LIANG Hong

Maternity and Child Healthcare Hospital of Chengdu, Chengdu, 610031 P.R.China

Objectives: To determine the medians of AFP and F-β-HCG of each week in pregnancy and the scope of high-risk groups via serological screening in a large sample of population. **Methods:** Serum marker test was performed for a total of 8341 pregnant women in their 14-21^{+6} weeks of pregnancy using time-resolved immunofluorometric assay system. The results were used for calculating the risks with special software. The high-risk pregnant women were identified, and antenatal diagnoses were made. The medians of AFP and F-β-HCG of each pregnant week were calculated by nonlinear weighted regression. **Results:** A total of 635 cases of Down's syndrome were identified on screening at a positive rate of 7.61% (635/8341). Eight infants were diagnosed with Down's syndrome, with one false negative case. The medians of AFP and F-β-HCG of each pregnant week were significantly different from the imbedded references on the screening software. All the nine cases were detected with the modified medians. **Conclusion:** Multi-center large-sample studies to establish the medians of AFP and F-β-HCG suited for serological screening of Down's syndrome in groups in different places are necessary for improved screening efficiency.

P8-7:

FOLLOW UP OF 28 CONGENITAL HYPOTHYROIDISM PATIENTS DIAGNOSED THROUGH THE HONG KONG GENETIC NEONATAL SCREENING PROGRAM 1984-2008

Albert C.F. Lam[1], May S.M. Fung[1], Anita M.W. Chow[1], Patricia Hung[2], Kitty C.K. Li[1], Ivan F.M. Lo[1], David K.H. Chan[1], Edgar W.L. Hau[1], Kai Man Kam[2], Stephen T.S. Lam[1]

[1]Clinical Genetic Service, Department of Health, HKSAR; [2]Public Health Laboratory Services branch, Centre for Health Protection, Department of Health, HKSAR

In March 1984, Newborn Screening for Congenital Hypothyroidism was commenced in Hong Kong. To evaluate the current health status of these patients and to ascertain whether thyroid disease is rare in these families. In 2007, we followed-up 28 families in which proband was diagnosed Congenital Hypothyroid at birth through the HK Genetic Neonatal Screening Program. Parents and siblings were also interviewed and thyroid function tests were performed. Total 20 female and 8 male patients, in which 1 patient with Agenesis, and 12 patients with Sublingual thyroid confirmed by thyroid scan at birth, 11 diagnosed with Dyshormonogenesis, and 4 diagnosed transient hypothyroid at 3 years old, and required no thyroxine replacement since. No patient was mentally retarded. Age ranged from 1 to 24 years old (mean 15), 12 families had family history of thyroid disease. 2 asymptomatic parents, 2 asymptomatic siblings, and 2 symptomatic close relatives from 6 different families had to be referred because of biochemical hypothyroidism. 1 transient hypothyroid patient whose thyroxine replacement was ceased at 3 years old, was now found to have much elevated TSH level, and was referred to Paediatricians for further management. 8 Congenital hypothyroid patients were also referred to Paediatricians because of persistent gross elevated TSH level. Amongst the 28 families, mean paternal TSH level was 1.64 mIU/L, FT4 level was 13.14 pmol/L. Mean maternal TSH level was 2.0 mIU/L, and mean FT4 level was 13.69 pmol/L. In Conclusion, all patients remained in good health, continual follow-up was shown to be essential for optimal management, and family history of thyroid disease was common in this cohort, the significance of which warrants future studies.

P8-8:

PREIMPLANTATION GENETIC DIAGNOSIS OF FIVE ROBERTSONIAN TRANSLOCATION COUPLES

Zhang Yue-ping, Han Jin-lan, Cao Xiang

Shanghai JiAi Genetics and IVF Institute, Shanghai, 200011, P.R.China

Objective: To survey the value of preimplantation genetic diagnosis (PGD) on Robertsonian translocation carriers. **Methods:** PGD was performed on five infertile couples with Robertsonian translocation. Each couple underwent one in vitro fertilization (IVF) cycle. Thirty-one embryos were biopsied 3 days after fertilization and 49 blastomeres were analysed by fluorescense in situ hybridization (FISH). Embryos with normal/balanced chromosomes were transferred the day after biopsy. Curettage was performed when miscarriage was diagnosed, and the karyotype of aborted conceptus was ascertained by G-banding technique. **Results:** Twenty-eight embryos got results after FISH. Among them 8 were balanced (28.57%) and 20 were abnormal (71.43%). The proportion of embryos with abnormalities due to the translocation was 50%, while the remaining 50% of embryos had abnormalities unrelated to the translocation. The incidence of mosaicism was 38.46% among 13 embryos in which diagnosis were made based on 2 balstomere FISH results. Seven embryos were transferred to 3 patients and one implanted successfully. The implantation rate was 14.29%. Ultrasonographic examination was given to the pregnant patient, but no fetal pole was found fifty days after transfer, so curettage was performed. The karyotype of aborted conceptus was 47,XY,der(14;21),+7. **Conclusion:** For infertile couples with Robertsonian translocations, PGD is a valuable screen for imbalance. But high incidence of mosaicism as well as abnormalities unconnected with the translocation may give rise to false negative results.

P8-9:

APPLICATION OF SCREENING FOR FETAL DOWN SYNDROME IN THE SECOND-TRIMESTER BY BAYESIAN SCORING SYSTEM

Liao Shi-xiu[1,2], Chen Han-ping[2], Huang Fei-fei[1], Yang Yan-li[1], Zhang Chao-yang[1], Wang Li[1], Wang Ying-tai[1]

[1]He Nan Medical Genetics Institute; [2]OG of Tongji Hospital Affiliated of Hua Zhong University of Science and Technology

Objective: The purpose of this study was to elevate the sensitivity of prenatal screening for Down syndrome, lower the false-positive rate and decrease invasive prenatal diagnoses. We have developed a new combining screening scheme using Bayesian scoring system, which was based on Bayesian theorem and the logistic regression model. **Methods:** We integrated three different screening strategies on maternal serum markers, genetic sonogram markers and maternal age by Bayesian Scoring System. These indexes of screening for Down syndrome were quantized on their sensitivity and specificity. The probability of fetal Down syndrome was obtained on the quantized indexes. When it was over two score, the fetus belonged to the group of high risk. The invasive prenatal diagnosis was done in those defined as risk cases. **Results:** In the trimester 3466 pregnant women accepted Bayesian Scoring System screening, 218 of which were detected positive for Down syndrome with a false positive rate of 5.04%, the positive rate was 6.28%. Fetal Down syndrome was diagnosed in 23 cases (prevalence 4.0‰). 21 cases were detected by our combining screening scheme; the screening positive rate was 91.30% (21/23). Pregnancies with positive screening results had a significantly higher risk of adverse outcomes and other defects than those with negative results. **Conclusions:** The Bayesian scoring system has been demonstrated to be a simple, efficient and specific method for prenatal screening Down syndrome.

P8-10:

THE CLINICAL STUDY OF FETAL CHROMOSOMAL ABERRATION INDUCED BY PAINT AND HAIR DYE: ADD 2 CASES OF FIRST REPORT

Yanhui Liu, Lifen Li, Yamin Wu

Medical Genetic Department, Tung Wah Hospital of Sun Yat-sen University, Guangdong Province, 523110

Chromosomes of one new born of 3 months and a fetus of 21 weeks were mutated. Their karyotypes were respectively 46,XX,del(2)(pter→q31) and 46,XX, t(4;12;15). Their parent have worked at paint and hair dye, and had dizziness, headache, insomnia and so on. Their chromosomes were all normal, but micronuclei of lymphocytes and plasma lead were higher, and WBC, platelet, HGB were lower. So, working long at paint or hair dye can cause poison and the noxious constituents can impact growth normally of early embryos by placenta and further lead to gene and chromosome mutation of embryos at last. So, people who plan to be pregnant especially for women should stay away from noxious environment to reduce ill pregnant happening.

P8-11:

MUTATION SPECTRUM OF THE G6PC GENE IN 79 CHINESE PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE 1A AND PRENATAL DIAGNOSIS IN 14 GSD 1A FAMILIES

Wenjuan Qiu, Je Ye, Lianshu Han, Xuefan Gu

Department of Pediatric Endocrinology and Metabolic Disease, Xinhua Hospital Affiliated to Shanghai Jiaotong University, Shanghai Institute for Pediatric Research

Glycogen storage disease type Ia (GSD Ia) is a recessively inherited disorder due to the deficiency of Glucose-6-phosphatase that results in impaired glycogen degradation and its accumulation in the lysosomes. We report here the molecular analysis of the G6PC gene performed on 79 Chinese patients with GSD Ia from 2003 to 2008. 17 mutations including 8 known mutations（727g>t, R83H, Q104X, R170X, W70X, 341del G, A264L, I341N）and 9 novel mutations(Y93X, W160X, L173P, L215P, G266D, G272R, S326P, 1147delA, IVS1+3A/T) have been identified. The 727G>T was the most frequent mutation, present as homozygote in 39.2 % of the patients and compound heterozygote in 43 % of the patients (allele frequency 60.76%). The R83H was the second frequent mutation, present as homozygote in 5 % patients and compound heterozygote in 22.8 % of the patients (allele frequency 17.72%). The nucleotide 1176 polymorphism was also analyzed. The 727g>t and R83H mutations demonstrated linkage disequilibrium with 1176C. The prenatal diagnosis of 14 GSD Ia families were performed and confirmed postnatal by the biochemical and molecular studies. Our finding suggest that the screening for the 727G>T and R83H mutations by restriction enzyme analysis combined with 1176 polymorphism linkage analysis is a simple, fast and accurate method for gene and prenatal diagnosis for GSD Ia in Chinese.

P8-12:

EFFECT OF FORMALDEHYDE TREATMENT ON THE RECOVERY OF CELL-FETAL DNA FROM MATERNAL PLASMA AT DIFFERENT PROCESSING TIMES

Yi Zhang, Zhenlin Hu, Qin Li, Ning Hui, Shuhan Sun

Department of Medical Genetics, The Second Military Medical University, Shanghai, China

Background: The effect of formaldehyde treatment on the recovery of fetal DNA from maternal plasma is controversial. The present study aims to evaluate the effect of formaldehyde and investigate the underlying mechanism. **Methods:** Blood samples from pregnant women were treated or not treated with formaldehyde, and processed at different times. Total and fetal DNA in plasma was quantified by real-time polymerase chain reaction. Death and lysis of blood cells were assayed by trypan blue exclusion test. Plasma DNase activity was determined by the radial enzyme-diffusion method. **Results:** Formaldehyde addition showed no effect on the percentage of fetal DNA in samples processed 6 h after blood collection. In samples processed at 36 h, formaldehyde addition inhibited blood cell lysis and nuclease-mediated DNA degradation, thus markedly decreasing the concentration of total DNA and increasing the recovery of fetal DNA. The median (interquartile range) percentage of fetal DNA increased from 4.6% (3.8–6.8%) to 13.1% (10.3–17.0%). **Conclusion:** The effect of formaldehyde on the percentage of fetal DNA in maternal plasma depends on processing time and is associated with prevention of cell lysis and inhibition of plasma DNase activity.

P8-13:

AN ANALYSIS ON THE GESTATION FINAL OUTCOME AND PATHOLOGIES OF THE CHROMOSOMAL ABNORMAL EMBRYO

Yao Hong, Liang Zhiqing, Chang Qing, Ge Xiaodong, Jangfeng, Xugang, Hubing, Wang Lu

Third Military University, Southwest Hospital, The Department of Prenatal Diagnosis Center (China, 400038)

Purpose: Inquiry into the existence value of the different chromosome abnormal fetal and supply the pregnant woman for choosing the gestation final outcome according to. **Method:** Trough abdomen amniocentesis to get the samples of the amniotic fluid or vein blood and check the chromosome using G-banding. **Result:** 34 of 300 fetal cases are chromosomes abnormal including 16 cases of 21 trisomy (2 mosaicism), 5 cases of 18 trisomy, 1 case of 13 trisomy, and 7 cases of sex chromosomal abnormality. Nineteen of these cases were autopsy by the patient consent. Two cases of nine 21-trisome merge cheilopalatognathus, 2 cases merge dodecadactylon abnormal, 3 cases merger heart abnormal. Two cases of four 18-trisome merge ventricular septal defect, 2 cases merge kidney and deformity of ureter abnormal. A case of 13-trisome are congenital heart disease and retinal dysplasia. In five sex chromosomes abnormality a case are 46, XX/46,XY with aedea abnormal. A fetal of t(1;3) is already two years old and the growth develop is normal. **Conclusion:** Trisomy chromosome embryo inheriting the increment performance of the material are often the intelligence lowly and companion internal organs abnormal. Most parents would choose to terminate the gestation. Sex chromosome abnormal fetes because of the number of its sex chromosome often express different degree of the sex development or no fertility mostly. The abnormal fetes through three super sound should be checked the chromosomes before the terminal pregnancy.

P8-14:

PCR/LDR/CAPILLARY ELECTROPHORESIS FOR NON-INVASIVE PRENATAL DIAGNOSIS OF BETA-THALASSAEMIA MUTATION

Ping Yi[1], Zhuqin Chen[1], Lili Yu[1], Jianxin Guo[1], Guodong Liu[2], Haichang Xie[1], Yuanguo Zhou[3], Li Li[1]

[1]Department of Obstetrics and Gynecology, Research Institute of Surgery, Daping hospital, Third Military Medical University, Chongqing, 400042; [2]Department of Molecular Biology, Research Institute of Surgery, Daping hospital, Third Military Medical University,Chongqing, 400042; [3]The Ninth Research Department, Research Institute of Surgery, Daping hospital, Third Military Medical University, Chongqing, 400042

Objectives: Analysis of fetal DNA in maternal plasma has recently been introduced as a new method for non invasive prenatal diagnosis. To date, the analysis of more subtle fetal-maternal genetic differences involving point mutations is more complex, as they are masked by the overwhelming presence of maternal DNA sequences in circulation. We have now investigated the feasibility of PCR/ligase detection reaction (LDR)/capillary electrophoresis for the detection of fetal point mutations such as IVS2 654(C→T) of beta-thalassaemia in maternal plasma DNA. **Methods:** The sensitivity of LDR/capillary electrophoresis was examined by quantifying the matched PCR products in the presence of vast excess of mismatched competitor templates, a situation mimic the detection of rare fetal mutations in the presence of excess maternal DNA.PCR/LDR/capillary electrophoresis was applied to detect specific mutations from experimental model at different sensitivity levels and one maternal plasma sample. **Results:** Our results demonstrated that the sensitivity of this approach for detecting low abundance single nucleotide mutation probably achieved to 1:10000. The approach was applied in maternal plasma DNA for detecting paternally inherited fetal IVS2 654(C→T) mutation and the result was the same to that of PCR/reverse dot blot (RDB) of amniotic fluid cell DNA. **Conclusions:** PCR/LDR/capillary electrophoresis has very high sensitivity to distinguish low abundance single nucleotide differences and probably detects paternally inherited fetal point mutations in maternal plasma. This approach can be extended for the non-invasive prenatal determination of other fetal loci.

P8-15:

EXPLORATORY DEVELOPMENT FOR DAZ GENE CONTENT IN MATERNAL PLASMA AS A NEW PRENATAL SCREENING INDICATOR OF DOWN'S SYNDROME

ZHANG Jian-Fang

Department of Gynecology and Obstetrics, Xijing Hospital, Fourth Military Medical University, No.15 Changlexi Road, Xi'an 710032,China

Objective: To discuss the feasibility of DAZ gene content in maternal plasma as a new prenatal screening indicator of Down's syndrome. **Methods:** Cell-free fetal DNA in maternal serum was isolated from 40 samples in pregnancy. Samples consisted of 2 women carrying male trisomy 21 fetuses, 18 carrying euploid male fetuses, 2 carrying female trisomy 21 fetuses, 18 carrying female fetuses. TaqMan probe real-time quantitative PCR was used to detect both DAZ gene on Y chromosome and β-actin gene of the mothers and the fetuses. **Results:** Down's syndrome pregnancies exhibit 2.5-hold higher levels of maternal serum cell-free fetal DNA compared with matched controls. None of the samples from women carrying female fetuses had detectable Y-chromosomal signals. **Conclusion:** Detection of cell-free fetal DNA in maternal serum by TaqMAN probe real-time quantitative polymerase chain reaction (real-time quantitative PCR) and on the basis of its amount is a potential prenatal screening marker for trisomy 21.

P8-16:

PRENATAL DIAGNOSIS IN 4 CASES WITH ISODICENTRIC Y CHROMOSOME

LKL NG[1], ET LAU[1], YKY KWOK[2], LYF TANG[1], CKM WONG[2], CP LEE[2], TN LEUNG[4], YF LO[5], R MARTINS[3], MHY TANG[1]

[1]Prenatal Diagnostic & Counselling Department, Tsan Yuk Hospital, Hong Kong; [2]Department of Obstetrics & Gynaecology, The University of Hong Kong, Hong Kong; [3]Centro Hospitalar Conde S. Januario, Macau; [4]HK Sanatorium & Hospital, Hong Kong; [5]Prenatal Diagnostic Clinic, Pamela Northern Eastern Hospital, Hong Kong

Among 14,939 prenatal samples (12,730 amniotic fluid and 2,209 chorionic villus) received for cytogenetic analyses from January 2004 to March 2008, four mosaic 45,X/46,X,idic(Y) were detected. One was referred because of maternal age and the other three for positive Down syndrome screening. In one case, initial diagnosis was non-mosaic 45,X in 30 cells analysed. Ultrasound examination showed male genitalia, and 45,X/46,X,+mar at a ratio 53:7 was found after re-examination of the slides. The other three cases showed similar karyotypes, with the proportion of marker chromosomes ranging from 11.8% to 63.3%. The marker chromosomes were identified by PCR and FISH to be isodicentric Y. Chromosome Y microdeletion by PCR were studied using a set of Y-specific sequence tagged site (STS) markers in the AZF region at Yq11. One case did not show Y microdeletion while AZFb,c regions were deleted in the other 3 cases, with intact, partial or deletion at region AZFa. All four cases resulted in the birth of phenotypically normal male babies.

P8-17:

PRENATAL DIAGNOSIS OF A CHIMERIC 46,XX/46,XY USING CYTOGENETIC AND MOLECULAR STUDIES

LYF TANG[1], L KL NG[1], K WONG[2], R MARTINS[2], MHY TANG[1], ET LAU[1]

[1]Prenatal Diagnosis & Counselling Department, Tsan Yuk Hospital, Hong Kong; [2]Prenatal Diagnosis Center, Department of Obstetrics & Gynecology, Centro Hospitalar Conde S. Januario, Macau

Chimerism is a very rare condition and natural incidence is unknown. It results from the fusion of two different zygotes to form a single embryo. A mixture of 46,XX/46,XY cell lines may be observed simultaneously if the zygotes are of different sex. Phenotypes of chimeric patient range from normal male or female to varying degrees of ambiguous external genitalia. We present a 46,XX/46,XY chimera observed during prenatal diagnosis for a 37 years old woman. Amniocentesis was performed at 17 weeks of gestation and the karyotype was 46,XX/46,XY [13:20]. Molecular study on cultured amniocytes and parental DNA using 19 autosomal polymorphic markers and 4 X-linked makers excluded maternal cell contamination. Results showed double paternal and single maternal genetic contributions in the fetus, indicating involvement of two sperms. Possible mechanisms are: 1. fertilization of an ovum and its second polar body with two sperms; 2. fertilization of a parthenogenetic ovum by two sperms. This pregnancy resulted in a healthy baby boy at term with normal external genitalia. Chromosomal study of placental tissue and fetal blood cultures confirmed findings of amniocytes. The ratio of XX to XY cell lines in placental tissue and fetal blood was 11:23 and 7:23 respectively.

P10-1:

THE ANALYSIS OF HEREDITY REGULATION IN A FIVE-GENERATION CONGENITAL SENSORY NYSTAGMUS PEDIGREE

Yong en Liang, Ying Wang

College of Life Science and Technology, Jinan University, Guangzhou, 510632, China

To classify and analyze the patent of congenital nystagmus, there were fifty seven members (thirty one males and twenty six females) in a five-generation family were investigated. According to the interview from this pedigree, there were seven males and two females were patient and nine females were heterogeneous. Although the appearances of heterogeneous were same as a normal as the symptom of congenital nystagmus was not occurred, the other symptoms of eyes and control nervous system were displayed, such as strabismus, cataract, glaucoma and abnormal of head position etc. It can explain that the relative gene belong this family was incompletely dominant. As a result, by concluding the analyzing, to eliminate the possibility of the other ways of heredity, can be ensure that the pattern of inheritance in this family was incompletely X-linked dominant congenital sensory nystagmus.

P10-2:

CLINICAL AND MOLECULAR CHARACTERIZATION OF 30 CHINESE PATIENTS WITH SEVERE FAMILIAL HYPERCHOLESTEROLEMIA

Luya Wang, Jie Lin, Shu Liu, Yinjie Xu, Junhui Xia, Shouchun Cao, Liwei Chen, Junliu, Xiaodong Pan

Department of Atherosclerosis, Beijing Anzhen Hospital, Capital Medical University, Beijing, 100029, China

Background: Familial hypercholesterolemia is a human monogenic disease and associated with an increased risk for atherosclerosis and premature coronary artery disease (CAD). It is often caused by certain mutations in the low density lipoprotein (LDL) receptor gene. Although there were a few studies on mutations of LDL receptor gene among Chinese FH patients, further investigation is certainly needed to characterize various forms of such mutations clinically and molecularly in a broader scale in this country consisting of 1/4 of world population. **Aim:** To characterize mutations in the LDL-R gene among 30 Chinese patients with clinical features of severe FH. **Method:** FH was diagnosed clinically according to LDL- cholesterol levels, tendon xanthoma, arcus cornealis, premature atherosclerosis and family history. B-mode ultrasound supplemented by Doppler was used to examine aortic/mitral valve structural alterations and carotid intima-media thickness (IMT). Patient coronary flow velocity reserve (CFVR) was assessed by transthoracic Doppler echocardiography and calculated as the ratio of maximal to baseline coronary velocities. Mutation analyses of LDL-R, ApoB100, PCSK9 and ARH genes were performed by Touch-down PCR and sequencing. LDL-R wild type gene was subcloned into OmicsLink mammalian cell expression vector with N terminal GFP tag. The LDL-R mutations were generated by site-directed mutagenesis using a QuikChange XL mutagenesis kit. The integrity of the constructs was confirmed by DNA sequencing. The WT and mutant LDL-R genes were transfected into HEK 293 cells. LDL-R function of transfected cells was analysed by flow cytometry after incubation in RPMI 1640 containing 10% LPDS (lipoprotein deprived serum) for 48 hours. **Result:** 30 probands were diagnosed as homozygotic FH according to clinical features. The mean age was 17.3 ± 9.4 year-old. Tendon xanthoma and arcus cornealis were noted in all patients. Both parents of 28 probands and single parents of 2 probands were proved to be hypercholesterolemia. The mean plasma LDL cholesterol level was 21.2 ± 5.8 mmol/L (ranged from 9.6 to 28.8 mmol/L) in this patient group. B-mode

ultrasound revealed aortic valve regurgitation and stenosis in 10 and 7 probands, respectively; and regurgitation of mitral valve in 14 patients. The IMT was increased to 0.12～0.21 cm in the 18 patients (<0.1cm). Carotid artery stenosis (range from 40 to 50%) was found in 6 patients. The mean of CFVR values was 2.30 ± 0.85 in this patients group with 16 of them ≤3.0 and 11 ≤2.0. Total 16 point mutations in the LDL-R gene in 19 probands (63%) were identified, including 12 missense (Y87C, C122Y ,C152R, C183Y, C210R, T383I, A459V, L561F, D601Y, D601N, A606T, and P664L), 3 nonsense (S565X, W462X, and Q12X), one splicing (G＞A splice donor mutation in intron 3) and 2 frame shift mutations (Gly157delA，Lys204delG). The flow cytometry analysis showed that the nonsense and frame shift mutation had more severe impairment in LDL binding and internalization than point mutations. 3 types of novel point mutations (A53V, G27c and I474V) in PCSK9 gene were detected in 7 probands (23%). The functional alterations of these mutations are currently under investigation. No mutations in LDL-R, R3500Q mutation in the APOB gene and 8 exons in ARH gene were found in other 4 patients. **Conclusion:** We identified 30 Chinese FH probands who have various clinical phenotypes revealed by B-mode ultrasound. Different mutations in LDL-R gene with functional impairment and in PCSK9 were found in 86% of them and further investigation is needed to identify the genetic defects in other 14 % of probands in this cohort.

P10-3:

INFANTILE CONVULSION CHOREOATHETOSIS SYNDROME IN A HONG KONG CHINESE FAMILY

Brian HY CHUNG*[1,2], CW FUNG*[1], Ping NG[1], Minghui ZHAO[1], Wanling YANG[1], Virginia CN WONG[1]

(*these 2 authors have equal contribution in the present study)

[1]Department of Paediatrics & Adolescent Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong; [2]Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario

Purpose: To describe the clinical features of a Hong Kong Chinese family with infantile convulsion choreoathetosis syndrome (ICCA) and the results of the preliminary genetic workup. **Method:** Medical records review was performed in our 2 index patients. Direct sequencing of the coding exons of SLC5A11 (Gene ID: 115584) was done on one of the index patients (CWP), his mother and normal sister. **Results:** Patient 1 (CWP) was a one -year old boy who presented to us with recurrent generalized tonic clonic convulsions since 6 months of age. He was treated with low dose valproate and remained seizure free. He had normal development. Patien t 2 (CWH) was a fifteen -year old boy. He had multiple seizures during an episode of viral illness at 11 months old and was then seizure free. Since the age of 10, he started to have paroxysmal kinesigenic dyskinesia (PKD) but was initially thought to have pseudoseizures. Once diagnosed, low-dose carbamazepine induced remission. He had normal intelligence. 4 other family members had infantile convulsions with complete remission after few years of age. They did not have PKD. 2 more had PKD without infantile c convulsion. They were not on treatment. The SLC5A11 gene was reported to be involved in ICCA in families in other populations. Direct sequencing of the 15 coding exons of SLC5A11 gene did not show any coding mutations or splicing junction changes in the affected family members, indicating that this gene may not be involved in this family. Further linkage study is in progress. **Conclusion:** Early identification of this epilepsy syndrome allows appropriate counseling of the prognosis of the infantile-onset epilepsy, which is usually benign and easy -to-treat. Paroxysmal kinesigenic dyskinesia, being another part of this syndrome might be misinterpreted as epilepsy.

P10-4:

DE NOVO ACVR1 G→A NUCLEOTIDE MUTATION IN A CHINESE PATIENT WITH FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

Ling-li Huang, Yue-qiu Tan, De-hua Cheng, Lu-yun Li, Guang-xiu Lu

Institute of Reproduction & Stem Cell Engineering, Xiang-ya School of Medicine, Central South University, Changsha, Hunan 410078, China

Fibrodysplasia ossificans progressiva (FOP) is a rare, severe disabling autosomal dominant disorder characterized by congenital malformations of the great toes and by postnatal progressive heterotopic ossification. A recurrent single nucleotide mutation 617 G→A (R206H) in the activin receptor Ⅰ A (ACVR1) gene, was once verified as the cause of FOP in all-classically affected individuals analyzed worldwide. Recently, a case of Japanese FOP patient with a novel mutation 1067G→A (G356D) in ACVR1 gene has been reported. Here, we present a 4-year-old FOP boy in Mainland China who carried a de novo single nucleotide mutation 617 G→A (R206H) of ACVR1 gene. He was noted with bilateral malformed great toes and restricted neck movement at birth. A hard subcutaneous mass was found on his right occipital region at 13 months age and then mutiple hard subcutaneous masses on his back at 4 years age. He gradually developed difficulty in mouth opening, lumber movement and major joints movement. He experienced many diagnostic exams including biopsy and some invasive interventions including muscular injection, which finally accelerated the progress of his condition. Analysis of AVCR1 gene in the patient and his parents revealed that a de novo heterozygous mutation 617 G→A (R206H) in ACVR1is responsible for the disorder. Our study further emphasizes the important role of mutation analysis for ACRV1 gene in early diagnosis and differential diagnosis of the disorder.

P10-5:

CLINICAL AND LABORATORY FEATURES OF SYSTEMIC LUPUS ERYTHEMATOSUS ASSOCIATED WITH SJOGREN'S SYNDROME IN A CHINESE POPULATION

Hai-Feng Pan, Wen-Xian Li, Qian Wang and Dong-Qing Ye

Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei, Anhui, 230032, PR China

Objective: To investigate the clinical and laboratory features of systemic lupus erythematosus patients associated with Sjogren's syndrome (SS) in China, as well as its similarities to, and differences from SLE patients without SS. **Methods:** A group of 542 consecutive unselected SLE patients was recruited in the present study. Diagnosis of SLE was made according to 1997 revised American College of Rheumatology (ACR) SLE criteria; SS was diagnosed using the American-European classification criteria. Clinical and laboratory parameters in SLE patients with SS (SLE-SS) were compared with those in SLE patients without SS (SLE-no SS). **Results:** SS was identified in 35 SLE patients (6.5%); the onset of SS preceded the development of SLE in 17 of them (48.6%). Compared with the SLE-no SS group (35.8±10.5 year), patients with SLE-SS (41.3±11.6 year) were significantly older (P=0.003), had a higher frequency of anti-Ro/SSA, anti-La/SSB and anti-dsDNA antibodies, but had a significantly lower frequency of renal involvement. **Conclusion:** SLE-SS may be a subgroup of patients with characteristic clinical and laboratory features. To improve the treatment outcomes in SLE-SS patients, more specific treatment should be applied based on those factors.

P10-6:

SPECTRUM OF SKELETAL DYSPLASIA SEEN IN CLINICAL GENETIC SERVICE, HONG KONG

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

Clinical Genetic Service, Department of Health, HKSAR Government

There is a wide spectrum of Skeletal Dysplasia seen in the Genetic Service in Hong Kong. Ranging from the commonly seen lethal type of Thanatophoric Dysplasia and its variant-the TD San Diego type (8), Perinatal lethal type of Hypophosphatasia(1), Campomelic Dysplasia (2), to the rarer lethal type of Osteogenesis imperfecta (3) and Boomerang Dysplasia (1). In the non-lethal group, skeletal dysplasia involving predominantly the metaphyses include Achondroplasia (25), Hypochondroplasia (15), and Metaphyseal Chondrodysplasia McKusick type (1). Skeletal dysplasia with major involvement of the spine include SED Congenita (10), Schwart-Jampel Syndrome(1), and SMD Kozlowski(1). Others include Chondrodysplasia Conradi-Hunermann type(1), Dyschondrosteosis (10), Spondylocostal dysplasia (2), Albright Hereditary Osteodystrophy(5), Cleidocranial Dysplasia (14), Tricho-rhino-phalangeal type I & II (12), Larsen Syndrome (7), Desbuquois Syndrome(2), and XL Hypophosphataemic ricket (7).

P10-7:

CLINICAL AND MOLECULAR FINDINGS OF MOWAT-WILSON SYNDROME IN CHINESE

Ivan FM Lo, Tony MF Tong, Stephen TS Lam

Clinical Genetic Service, Department of Health, 3/F Cheung Sha Wan Jockey Club Clinic, 2 Kwong Lee Road, Shamshuipo, Kowloon, Hong Kong SAR

Mowat-Wilson syndrome (MWS) is a relatively new syndrome. It was first described by Mowat et al. in 1998, in six patients who shared features of Hirschsprung's disease, microcephaly, mental retardation, and dysmorphic facial features. In 2001, loss-of-function mutations of the *ZFHX1B* gene were found to be the cause of this syndrome. The gene product, Smad interacting protein-1, is a transcription factor that belongs to two-handed zinc finger/homeodomain family and plays an important role in the development of neural crest. Here we describe the clinical and molecular findings of six local Chinese patients (including a pair of twins) from five families with this syndrome. They shared characteristic facial dysmorphism, marked developmental delay and mental retardation, epilepsy of early onset, and ataxia. However, none of our patients had Hirschsprung's disease. They showed a happy disposition and friendliness to strangers, which, together with the ataxia, had led to the misdiagnosis of Angelman syndrome in a few patients. They were all found to carry heterozygous mutations of the *ZFHX1B* gene. The mutations are heterogeneous, including multiple exon deletions and small insertion/deletions.

P11-1:

FUNCTIONAL STUDY OF *STE7* GENE IN THE BIOSYNTHESIS OF EXOPOLYSACCHARIDE PRODUCED BY *STREPTOMYCES* SP.139

Liping Bai[1], Junjie Shan[2], Yang Zhang[1], Ren Zhang[1,3] and Yuan Li[1]

[1]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing China; [2]Institute of Pharmacology and Toxicology, Beijing China; [3]School of Biological Sciences, University of Wollongong, NSW 2522, Australia

Ste7 is one of the genes located in the Ebosin biosynthesis gene cluster of *Streptomyces* sp. 139 but its function in the pathway is not known. Homology search indicated its likeness of coding for a glycosyltransferase for nucleotide sugar transfer. The gene was disrupted with a double crossover via homologous recombination and the resultant mutant verified by Southern blot and PCR analyses. Compared with Ebosin, the monosaccharide composition of exopolysaccharide (EPS-m) produced by the mutant *Streptomyces* sp.139 (*ste7*$^{-}$) was found quite different; although containing the same sugar components the proportions changed significantly. Six out of eight sugar components were reduced in proportion but most noticeable were the drops (about 80%) in glucose and fucose contents. The other two, galactose and rhamnose were increased in proportion. Gene complementation largely reversed the composition changes including the decrease of fucose but had little effect on glucose content. The antagonist activity of EPS-m for IL-1R *in vitro* was remarkably lower than Ebosin at both tested concentrations. Compared with EPS-m this bioactivity of EPS-c produced by the complemented strain was significantly higher and it was even higher than Ebosin at the lower concentration (at 4.5μg/mL). These results have demonstrated the functional involvement of *ste7* gene in the biosynthesis of Ebosin mostly probably by encoding a glycosyltransferse for fucose transfer.

P11-2:

A NOVEL RNA-SPLICING MUTATION IN TRAPPC2 GENE CAUSING X-LINKED SPONDYLOEPIPHYSEAL DYSPLASIA TARDA IN A LARGE CHINESE FAMILY

Hong Guo, Xueqing Xu, Kai Wang, Bo Zhang, Yan Wang, Yun Bai

Department of Medical Genetics, Third Military Medical University, Chongqing, PR China

X-linked spondyloepiphyseal dysplasia tarda is an X-linked recessive disease, characterized by disproportionately short stature and degenerative osteoarthritis. SEDT is caused by the TRAPPC2 gene, which spans a genomic region of ~20 Kb in Xp22. In a large Chinese SEDT family, we screened all 6 exons of TRAPPC2 gene and identified a novel RNA-Splicing mutation (IVS4+1A>G). We also demonstrated that the mutation induced splice pattern change from AT/AC to GT/AG. As a result, the first seven nucleotides of exon 5 were spliced out from the transcript. The prediction of the amino acid sequence showed that the seven nucleotides deletion of the transcript caused frame shift and led to the translation termination in advance, which caused two alpha helixes of the protein loss. The results of our study expand the spectrum of the gene mutations associated with SEDT, and will help to further elucidate the role of this protein in the etiology of this form of osteochondrodysplasia.

P11-3:

A HAPLOTYPE VARIANT OF HMYH IN CHINESE PATIENTS WITH COLORECTAL CANCER: ENCODING A HMYH PROTEIN DUALLY LOCALIZED IN THE NUCLEUS AND MITOCHONDRIA

Huimei Chen, Lizhi Xu, Qiufeng Qi, Yanweng Yao, Ming Zhu, Yaping Wang

Department of Medical Genetics, Nanjing University School of Medical, Nanjing, China

The human MutY homolog (hMYH) is a DNA glycolsylase involved in the excision repair of oxidative DNA damage. We previously demonstrated that a haplotype variant c.53C>T/c.74G>A of *hMYH* increased high risk for gastric cancer in Chinese. To determine whether this haplotype variant of *hMYH* involved in colorectal carcinogenesis, we performed a case-control study in 138 colorectal cancer (CRC) patients and 243 controls in Chinese. We detected the frequency of haplotype variant (heterozygote) was higher in CRC patients than that in controls (P = 0.02, OR = 5.06, 95% CI = 1.26–20.4). As the variant is predicted to generate missense mutations, which were mapped to N-terminal functional mitochondrial targeting sequences (MTS), we further transfected *hMYH* cDNA with a flag epitope tag into COS-7 cells to examine the subcellular localization of the mutant protein. Immunofluorescence showed that the mutant protein of hMYH dually localized in nucleus and mitochondria, in contrast to that the wild-type protein only in mitochondria. These results suggested the haplotype variant of *hMYH* would affect protein mitochondria localization and could decrease cell repairing function of mtDNA oxidative damage. It might be responsible for the increase risk for CRC.

P11-4:

GENE MUTATION ANALYSIS IN PATIENTS WITH PROPIONIC ACIDEMIA

HU Yu-hui, HAN Lian-shu, YE Jun, QIU Wen-juan, ZHANG Ya-fen, YANG Yan-ling, LIU Li, MA Hong-wei, GAO Xiao-lan, GU Xue-fan

Shanghai Institute of Pediatics, Xinhua Hospital, Medical College of Shanghai Jiaotong University, Shanghai, 200092, China

Objective: Propionic acidemia is a common organic acidemia, caused by deficiency of propionyl-CoA carboxylase (PCC), which catalyze the carboxylation of propionyl-CoA to D-methylmalonyl-CoA.PCC is a dodecameric enzyme of α-PCC and β-PCC subunits, nuclearly encoded by genes PCCA and PCCB, respectively. Mutation in either gene cause Propionic acidemia, the PCCA gene is located on chromosome 13q32 with 24 exons and the PCCB gene is located on chromosome 3q13.2-q22 with 15 exons. In this study, we analyzed gene mutations of 11 PCCA and PCCB deficient patients from China and to explore the possible mutation spectrum of Chinese patients. **Methods:** All 39 exons of PCCA and PCCB genes in 11 unrelated Chinese PA patients were analyzed by polymerase chain reaction (PCR) and direct sequencing. Genomic DNA was extracted using Phenol-Chloroform method from the peripheral blood leukocytes of each patient. PCR amplification products were checked by 1.8% agarose gel electrophoresis and were subsequently sequenced with ABI 3700 Automated DNA Sequencer. **Results:** We identified 12 PA mutations, 7 affecting the PCCA gene, 5 affecting the PCCB gene, including 9 novel mutations and 3 previously reported mutations. Three missense mutations (1079T>G, 1102G>C and 1850T>C) and one short deletion (1863delA) were found in α-PCC subunit while three missense mutations (484G>A, 601G>A and 1253C>T) and two short insertion-deletions (167-179del13ins1, 560-561delCCinsT) were found in β-PCC subunit. The 167-179del13ins1 change was identified in two homozygous PA patients, with allelic frequency of 40% in β-PCC subunit deficiencies. **Conclusion:** Twelve mutations were found in 11 Chinese PA patients including nine novel mutations. No mutation is predominant in Chinese PCCA and PCCB deficient patients.

P11-5:

ANALYSIS OF GENE MUTATIONS IN CHINESE PATIENTS WITH METHYLMALONIC ACIDURIA AND HOMOCYSTINEMIA

WANG Fei, HAN Lian-shu, HU Yu-hui, YANG Yan-ling,,YE Jun, QIU Wen-juan, ZHANG Ya-fen, GAO Xiao-lan, WANG Yu, GU Xue-fan.

Shanghai Institute for Pediatric Research, Xinhua Hospital, Shanghai Jiaotong University School of Medical, Shanghai 200092, China

Objective: Methylmalonic acidemia and homocystinemia, cblC type, is the most common inborn error of cobalamin metabolism. The present study aimed to identify the mutation types of MMACHC gene and analyze the genotype-phenotype correlations in Chinese patients. **Mothods:** The entire coding region of MMACHC gene was screened by polymerase chain reaction (PCR) combined with DNA direct sequencing in 28 Chinese methylmalonic acidemia and homocystinemia patients. **Results:** Ten mutations were identified in 27 of 28 Chinese patients. Four mutations had been reported, which were 609G>A (W203X), 217C>T (R73X), 271dupA (R91KfsX14), 394C>T (R132X). Six were novel mutations, which were 1A>G, 365A>T, 658_660delAAG, 301-3_327del 30, 567_568insT, and 625_626insT. The 609G>A (W203X) is the most common mutation, which was detected in 30 of 56 alleles (53.6%), including 10 homozygous mutations and 10 heterozygous mutations. In addition, three gene polymorphisms were detected, namely, -302T>G (rs3748643), -234A>G(rs3728644), and 321G>A(rs2275276). These mutations include missense mutations, nonsense mutations, duplication, deletions, and insertions. **Conclusion:** In this study, we found a part of gene mutations spectrum in Chinese patients with methylmalonic acidemia and homocystinemia, in which the 609G>A(W203X) may be the hot spot of MMACHC gene. This would help for the prenatal diagnosis and gene screening programs of methylmalonic acidemia and homocystinemia.

P11-6:

THE ROLE OF SH3BP2 IN CHERUBISM

Ying Hu, Cuiying Li, Jinsong Wang

School of Stomatology, Capital Medical University, Beijing, China

Cherubism is a rare hereditary multilocular cystic disease of the jaws with incomplete penetrance characterized by extensive pathological bone remodeling within the mandible or maxilla due to heterozygous germline mutations in the gene that encodes the adapter protein (SH3-domain binding protein 2, SH3BP2). Recent studies have revealed 8 point mutations in this gene on chromosome 4p16.3 in both cherubism families and non-familial cases. These mutations described thus far in patients with cherubism, P418R, P418L, P418H, G420E, G420R, R415P, R415Q and D419G, all occur within a six amino acid region (amino acids 415-420) encoded by exon 9.In order to reveal how these different mutations affect the function of SH3BP2, we make the mRNA secondary structure prediction and try to discover the function change caused by these mutations. Good resource and software for predicting the mRNA secondary structure on the RNA Structure Database website at the Belozersky Institute of physico-chemical biology at Moscow State University are available and applied to analyze the data. The obvious distinction was detected for P418R and P418L mutations. The length of base paired region was shorter and the internal loop bulged more severely at the mRNA for the patients compare with the normal subjects. The cherubism symptoms of patients with these two mutations are more serious accordingly, and lesions can not cease even if patients are over 40 years old. In a word, the missense mutations P418R and P418L may play crucial role in the identification of cherubism.

P11-7:

OF HUMANS AND *DROSOPHILA*, GENETICS OF OPTIC ATROPHY

Taosheng Huang

Department of Pediatrics, Division of Human Genetics, *Department of Pathology & Laboratory Medicine, [3]Department of Developmental and Cell Biology, University of California, Irvine, CA*

Optic atrophy gene 1 (OPA1) is a nuclear gene encoding a mitochondrial protein. Mutation of OPA1 is the most common cause for autosomal dominant optic atrophy (DOA). This condition primarily affects eyes, and is characterized by gradual vision loss, color vision defects, and temporal optic pallor. Previous studies have shown that OPA1 is ubiquitously expressed and serves as a gatekeeper for cytochrome-c release, and is important in mitochondrial fusion and ATP production. We have recruited a large number of patients including 6 large families to our study. A subset of the patients carries the R445H mutation, which causes loss of vision and hearing. Our electrophysiological analysis shows that this mutation causes asynchronous cochlear conduction and cochlear implants provide remarkable results, restoring hearing. In order to understand the molecular mechanism by which *OPA1* mutations cause optic atrophy and to facilitate the development of an effective therapeutic agent for optic atrophies, we analyzed phenotypes in the developing and adult *Drosophila* eyes produced by mutant *dOpa1 (CG8479)*, a *Drosophila* ortholog of human *OPA1*. Heterozygous mutation of *dOpa1* by a P-element or transposon insertions causes no discernable eye phenotype, whereas the homozygous mutation results in embryonic lethality. Using the powerful *Drosophila* genetic techniques, we created eye-specific somatic clones. The somatic homozygous mutation of *dOpa1* in the eyes caused rough (mispatterning) and glossy (decreased lens and pigment deposition) eye phenotypes in adult flies; this phenotype was reversible by precise excision of the inserted P-element. Furthermore, we show the rough eye phenotype is caused by the loss of hexagonal lattice cells in developing eyes, suggesting an increase in lattice cell apoptosis. In adult flies, the *dOpa1* mutation caused an increase in reactive oxygen species (ROS) production as well as mitochondrial fragmentation associated with loss and damage of the cone and pigment cells. We show that superoxide dismutase 1 (SOD1), Vitamin E and genetically overexpressed human SOD1 (hSOD1) is able to reverse the glossy eye phenotype of dOPA1 mutant large clones, further suggesting that ROS play an important role in cone and pigment cell death. Since

OPA1 is ubiquitously expressed, suggesting that mutations in OPA1 cause mitochondrial dysfunction and affect multiple organs. The grossly normal structure eye phenotypes in heterozygous mutant *Drosophila* do not imply normal function. In this study, we demonstrate that heterozygous *dOpa1* mutation perturbs the normal ERG profile of the *Drosophila* compound eye in an age-dependent manner. These abnormalities were partially reversible through the administration of antioxidants. dOpa1 mutation causes a progressive loss of cardiac function and poor tolerance to high rate pacing in an age-dependent manner. This defect was not affected by antioxidant. The skeletal muscles were normal in heterozygous mutant. However, under stress, heterozygous mutants reduced physical fitness. Our results suggested that heterozygous mutation of *dOpa1* causes multiple organ abnormalities in an age-dependent manner. Some organs damage might be mediated through increased ROS production. Our results showed that mutation of *dOpa1* causes increased production of ROS and poor tolerance to stress. We will examine superoxide dismutase (SOD) activity and mitochondrial respiration rate in our $dOpa1^{+/-}$ flies. Finally, we will test if antioxidants can rescue the shortened lifespan phenotype. This study provides novel insights into the pathogenesis of optic atrophy and will assist in the development of novel therapies to prevent vision loss. The clinical relevance of the research is strengthened by studying an animal model and human subjects in parallel. Many degenerative retinal diseases share similar clinical features and therefore, the data from this study may be extended to those diseases.

P11-8:

GAP JUNCTION PROTEIN ALPHA 12 GENE MUTATION AND UPD1 IN TWO CHINESE PATIENTS WITH PELIZAEUS-MERZBACHER-LIKE DISEASE

Yuwu Jiang[1], Jingmin Wang[1], Yueying Wang[2], Huifang Wang[1], Tianjian Chen[2], and Xiru Wu[1]

[1]Department of Pediatrics, Peking University First Hospital, No. 1, Xi An Men Street, West District, 100034, Beijing, China; [2] Department of Medical Genetics, University of South Alabama, Mobile, AL 36688, USA

Pelizaeus-Merzbacher disease (PMD) and Pelizaeus-Merzbacher-like disease (PMLD) are hypomyelinating disorders of the central nervous system caused by mutations in the PLP1 and GJA12 genes, respectively. Uniparental disomy (UPD) is defined as the presence of a chromosome pair, in a diploid individual, that derives from only one parent. To date, studies of the GJA12 gene mutation and associated with Chromosome 1 UPD both have not been reported in PMLD patients. In this study, GJA12 gene mutations in two Chinese PMLD patients and one of them with paternal UPD for chromosome 1 were identified. The patient 1 harbored a homozygous frameshift mutation at c. 216delGinsAA (p. P73fsX106) in the GJA12 coding region. Her father was heterozygous for the mutation, but her mother was homozygous for the wild type allele. Haplotype analysis of the entire chromosome 1 of the patient revealed that this chromosome was exclusively derived from her father. Non-maternity inheritance was confirmed by allelotype analysis of autosomes other than chromosome 1. The GJA12 gene is located on chromosome 1q41-42 and falls within the region of paternal isodisomy on the q arm. Thus, a novel homozygous frameshift mutation p. P73fsX106, caused by paternal UPD for chromosome 1, was identified in patient 1 with PMLD. Patient 2 was found a homozygous missense mutation at c.138C>G (p.I46M). Two novel mutations of GJA12 c.216delGinsAA (c.P73fsX106) and c.138C>G (p.I46M) were identified in two Chinese patients with PMLD. The c.216delGinsAA (p.P73fsX106) was caused by paternal UPD for chromosomal 1.

P11-9:

IMAGE-BASED HIGH-THROUGHPUT POPULATION SCREENING FOR ERYTHROPOIETIC PROTOPORPHYRIA

Kin-Chong Lau and Ching-Wan Lam

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong

Partial deficiency of ferrochelatase activity leads to excessive accumulation of cellular protoporphyrin and causes erythropoietic protoporphyria (EPP). Based on the auto-fluorescence of protoporphyrin, an initial diagnosis of EPP can be established by the presence of fluorescent red cells (fluorocytes) in a fresh blood film viewed under ultraviolet light. For high-throughput analysis, we have improved this method by using an automatic image acquisition platform for examining fluorocytes in peripheral blood. In this pilot study, three EPP patients were diagnosed and 4000 normal individuals were screened for EPP by this method. The image acquisition is easy-of-use which under automated operations of excitation, focusing, detection and data analysis. Quality image and semi-quantitative fluorescence measurement of fluorocytes can be generated in a single step. The platform can image more than two hundred 96-well micro-plates in approximately 10 hours. Our results showed that the method can distinguish the overt cases from asymptomatic carriers. Since fluorocytes are present in umbilical cord blood of EPP patients, this high-throughput method can be potentially used for newborn screening of EPP.

P11-10:

DNA-BASED DIAGNOSIS OF ERYTHROPOIETIC PROTOPORPHYRIA IN PATIENTS PRESENTING WITH "ACUTE PORPHYRIC" SYMPTOMS

Kin-Chong Lau and Ching-Wan Lam

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

Erythropoietic protoporphyria (EPP) is a rare inherited disorder of haem biosynthesis and skin photosensitivity resulting from a partial deficiency of ferrochelatase (FECH) activity. Previous studies showed that the frequency of a low expression allele (IVS3-48C) of the FECH gene control the penetrance of EPP. To our knowledge, EPP were rarely reported in Chinese population. In this study, we performed a population screening of the low expression allele by restriction analysis. In addition, we performed molecular investigations of two EPP families. Direct DNA sequencing of the PCR products encompassing all the exon and intron boundaries of the FECH gene of the probands showed two maternally inherited point mutations. Each of which was trans co-inherited with the low expression allele in the patients. The allele frequency of IVS3-48C polymorphism in Hong Kong populations was determined to be 28%. This allele frequency is much higher than that of European populations. With such a high population frequency of the low-expression allele, we suggest that patients with EPP are being underdiagnosed in Chinese population.

P11-11:

MOLECULAR INVESTIGATIONS OF IDURONATE-2-SULFATASE MUTANTS RELATED TO SEVERE MUCOPOLYSACCHRIDOSIS TYPE II

Kin-Chong Lau and Ching-Wan Lam

Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong

Mucopolysacchridosis type II (MPS II, Hunter disease) is a X-linked lysosomal storage disease resulting from partial deficiency of iduronate-2-sulfatase (IDS) due to various mutations. In two severely affected patients, *IDS* missense mutations, c.1016T>C (novel) and c.1016T>G (known) were identified predicting the substitution of an ambivalent cyclic proline and a hydrophilic arginine respectively for the hydrophobic leucine at residue 339. We hypothesized that residue Leu339 may be functionally critical. To verify the single nucleotide change to be pathogenic, we performed a study for the two mutations by in-situ mutagenesis, *in vitro* expression, and functional analysis. Transient expression revealed that both the missense variants had stable mRNA but their residual enzyme activities remained less than 2.5 % of normal level. Western blot analysis showed that both the missense variants synthesized the precursor form but had reduced mature forms of IDS. We concluded the novel mutation p.L339P is a disease-causing mutation affecting maturation of the protein.

P11-12:

CLONING AND CHARACTERIZATION OF A NOVEL RIBONULCEASE A SUPERFAMILY MEMBER—HUMAN RIBONUCLEASE 9

Guizhi Cheng, Fang Li

Department of Immunology, Dalian Medical University, Dalian, China, 116044

In this present study we cloned human ribonulcease (RNase) 9 cDNA from human adult epididymis RNA, prepared recombinant human RNase 9 from E.coli. In vitro we examined the ribonucleolytic activity of recombinant human RNase 9 against yeast tRNA, and the antibacterial activity of recombinant human RNase 9 against *E.coli* with the colony-forming unit (CFU) assay. Our results revealed that recombinant human RNase 9 exhibited antibacterial activity against E.coli in a concentration, time dependent manner and didn't exihibit detectable ribonucleolytic activity even at micromolar concentration. These results suggest that human RNase 9, a RNase A superfamily member is bactericidal, but not ribonucleolytic, and may be involved in host defense.

P11-13:

THE LOCATION OF INMAP IN SODIUM NORCANTHARIDIN-TREATED MOUSE ORGANS

Qianjin Liang, Yang Luo, Taoran Dong

College of Life Sciences, Beijing Normal University, Beijing Key Laboratory, Beijing 100875, P.R. China; Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R. China; Key Laboratory of Molecular and Cellular Genetics and Crop Breeding (BNU), College of Life Science, Beijing Normal University, Beijing, P.R. China

This work was supported by National Natural Science Foundation of China grants No30771101 and 30470875 and the Foundation granted by Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education of China. Sodium norcantharidin is one of the derivatives of catharidin. Previous experiments showed that the main pharmacological effects of Sodium norcantharidin is its function to inhibit cancer cell growth and division, therefore, sodium norcantharidin can be used as an effective anti-cancer drugs. INMAP (Interphase Nucleus & Mitotic Apparatus Protein) is a new kind of microtubule binding protein which was found by our group. The function of INMAP is closely related to mitosis. Over-expression of INMAP can lead to failure of cytokinesis and cause the formation of abnormal spindle, thereby affecting cell division. The purpose of this experiment is to study whether the location and expression of INMAP in mouse organ tissues will be effected after intraperitoneal injection of sodium norcantharidin. 16 Kunming white mice were randomly divided into four groups, each group with four. One group was the control which was injected with 0.9% NaCl. The remaining three groups were experimental groups, which were given intraperitoneal injection of concentration of 2 mg / kg, 6mg/kg and 10 mg / kg of sodium norcantharidinn respectively. In this experiment, mice received injection every two days and were stopped injection after one week. Then frozen section was applied to the brain, muscle, liver and kidneys tissues and indirect immunofluorescent analysis was applied. The results showed that INMAP positioning in the experimental group and control group did not have significant differences. So it can be assumed that sodium norcantharidin does not affect the process of cell division by affecting INMAP. However, the results also showed that locations of INMAP in various organs were different: in the brain tissue, INMAP mainly located in the nucleus, in some cells

INMAP can also be observed to locate around the nuclear membrane. In muscle tissue, the location of INMAP was found along the muscle fibers, but this result needs to apply repeat experiment to test its reliability. In liver and kidney tissues, INMAP mainly distributes in cytoplasm. All the results still need more repeat experiments to verify their reliabilities, and at least they may reflect tissue special diversity of protein distribution.

P11-14:

A GENETIC ANALYSIS OF THE POISONOUS EFFECT OF *SOLANUM ROSTRATUM* EXTRACT ON THE DEVELOPMENT OF *CAENORHABDITIS ELEGANS*

Qian-jin LIANG, Peng JIANG, Fan FENG

1. Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R. China; 2. Key Laboratory of molecular and Cellular Genetics, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R. China

This work was supported by National Natural Science Foundation of China grants No30771101 and 30470875 and the Foundation granted by Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education of China. The extract of *Solanum rostratum* was used to treat *Caenorhabditis elegans*. Then, the effect of diffenent *Solanum rostratum* extract concentrations on *C.elegans* was assessed by survival rate detection, protein SDS-PAGE, esterase isoenzyme analysis and DNA damage analysis in order to understand how *Solanum rostratum* reacts as a harmful alien invasive species. The results showed that: the growth and development of *C. elegans* were inhibited by the extract of the *Solanum rostratum* diluted by 100 times and deaths were induced. At the same time SDS-PAGE displayed that the extract could affect the protein expression in *C. elegans*; the activity of the esterase isoenzymes dropped down sharply in the experimental group; however, DNA degradation was not found. As is mentioned above, the overall harmful effect of the extract from *Solanum rostratum* verified is on protein expression spectrum, and esterase isozyme activity were changed, and the survival rate of *C. elegans* is decreased under the treatment of high concentrations. Therefore, *Solanum rostratum* is with biggish invasive fatalness, and strategies should be put forward to prevent its invasive harm. (April 10, 2008)

P11-15:

PHOSPHORALATION IDENTIFICATION AND FUNCTIONAL ANALYSIS OF INMAP, A NOVEL MICROTUBULE PROTEIN

Qianjin Liang[1,2], Yan Lei[1], Enzhi Shen[1], Qian Liu[1,], Le Sun[3], Yongchao Wang[1]

[1]Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, P.R. China; [2]Health Science Institute of Beijing Normal University, Beijing 100875, China; [3]Welson Pharmaceuticals, Haidian, Beijing 100085, PR China

This work was supported by National Natural Science Foundation of China grants No30771101 and 30470875 and the Foundation granted by Key Laboratory of Cell Proliferation and Regulation Biology of Ministry of Education of China. INMAP, discovered by our group, is a 40 kDa microtubulin related protein that distributes as dots in nucleus during interphase and associated with mitotic spindle in mitotic phase. The pattern is similar to the location of NuMA and NuSAP, two well studied proteins. In order to identify the domains of INMAP contributing to its location and function, the mutant HeLa cell strain expressing INMAP lacking of carboxyl and amidogen-terminal was constructed. The study indicated that INMAP was modified and phosphorylated by the relevant cyclin-dependent kinases during mitotic phase. There are predicted p34cdc2 consensus phosphorylation sites in INMAP. To test if predicted phosphorylation sites are necessary for INMAP's specific interaction with mitotic spindle, we introduced mutations into INMAP that converted the phosphorylation site from serine residue into alanine residue, and subsequently measured the change of the location of INMAP, cell cycle and the capacity of cell proliferation. In the HeLa cells expressing site-directed mutant INMAP, the adhesion ability declined, and the dephosphorylated INMAP may affect the expression of gap-link protein or disturb microtubule polymerization or depolymerization; the decrease of cell growth was induced by the inhibition of the linkage between INMAP and spindle. As a result, HeLa cells were arrested in mitotic phase and the capacity of cell proliferation dropped down. The key point we have held is that the interaction of INMAP with spindle microtubules may be controlled by cell cycle-dependent phosphorylation. （April 18, 2008）

P11-16:

PTPN11 MUTATIONS IN LEOPARD SYNDROME: REPORT OF FOUR CASES IN TAIWAN

I-Shou Lin, Sheau-Chiou Chao, Shio-Jean Lin

Departments of Pediatrics and Dermatology, National Cheng Kung University & Hospital, No. 138, Sheng Li Road, Tainan, Taiwan 704, R.O.C.

The LEOPARD syndrome (LS), also known as cardiocutaneous lentiginosis syndrome or multiple lentigines syndrome is a complex dysmorphogenetic disorder transmitted as an autosomal-dominant trait. The acronym LEOPARD was first introduced by Gorlin et al. in 1969 as the name to recall the syndrome: L, lentigines; E, electrocardiographic conduction abnormalities; O, ocular hypertelorism; P, pulmonary stenosis; A, abnormalities of genitalia; R, retardation of growth; and D, deafness (sensorineural). Noonan syndrome (NS), a heterogeneous disorder, shared many features clinically with LS. Noonan syndrome has been linked to markers on chromosome 12q24 and missense mutations in the PTPN11 gene, located in 12q24, were identified in 50% of the patients of NS. Interestingly, different locations of missense mutation in the PTPN11 gene had also been identified in cases of LS. Besides, the high penetration rate of the mutation, up to over 90%, was also the feature of the LS. We reported here four Taiwanese patients of LEOPARD syndrome. All four cases were found to have point mutations in PTPN11 gene. Two male patients were identical twin, hypertrophic cardiomyopathy were noted during neonatal stage. Lentigines developed later in childhood. Molecular analysis was performed and mutation T438M was identified. Two other cases were both male presenting with short stature and characteristic skin manifestation. One is 16 y/o boy who carry G464A point mutation. With our experience, the LS could be identified by general pediatricians noting the lentigines of the patients. The molecular study could further confirmed the diagnosis and provide information for genetic counseling.

P11-17:

CLINICAL AND MOLECULAR FINDINGS OF EIGHT PATIENTS WITH CARDIO-FACIO-CUTANEOUS SYNDROME

Ivan FM Lo[1], Yoko Aoki[2], David KH Chan[1], Tony MF Tong[1], Stephen TS Lam[1]

[1]Clinical Genetic Service, Department of Health, 3/F Cheung Sha Wan Jockey Club Clinic, 2 Kwong Lee Road, Shamshuipo, Kowloon, Hong Kong SAR; [2]Department of Medical Genetics, Tohoku University School of Medicine, Japan

Cardio-facio-cutaneous syndrome (CFC) is a clinically distinct but genetically heterogeneous multiple congenital anomaly/mental retardation (MCA/MR) syndrome. The clinical phenotype overlaps with those of Noonan syndrome and Costello syndrome, and includes postnatal growth retardation, characteristic craniofacial dysmorphism, ectodermal features of sparse hair, follicular hyperkeratosis and acanthosis nigricans, significant developmental delay and mental retardation, hypertrophic cardiomyopathy, and ophthalmological abnormalities. Genetic defects have been identified in *KRAS*, *BRAF*, *MEK1* and *MEK2* genes, which are all players in the RAS-ERK signaling pathway important for the control of cellular proliferation, growth and death. The same signaling pathway is also implicated in the pathogenesis of Noonan syndrome, Costello syndrome, and Neurofibromatosis type I, hence the phenotypic overlap among these disorders. Here we describe the clinical and molecular findings of eight CFC patients in Hong Kong. Seven patients had molecular studies performed in Japan. *BRAF* mutations were found in five patients, two being novel mutations. *MEK2* mutation was found in one patient. One patient did not have the molecular defect identified yet.

P11-18:

SHOX MUTATIONS IN CHINESE PATIENTS WITH SHORT STATURE

Ivan FM Lo, Tony MF Tong, Edgar WL Hau, Albert CF Lam, Stephen TS Lam

Clinical Genetic Service, Department of Health, 3/F Cheung Sha Wan Jockey Club Clinic, 2 Kwong Lee Road, Shamshuipo, Kowloon, Hong Kong SAR

The short stature homeobox-containing gene (*SHOX*) gene is located in the pseudoautosomal region on X/Yp. It is highly conserved across species from mammals to fish and flies. It encodes a transcription factor that is expressed in trabecular bone cells and bone marrow stromal fibroblasts. Haploinsufficiency of *SHOX* contributes significantly to the short stature phenotype and the skeletal abnormalities in Turner syndrome patients. Isolated *SHOX* mutations, mostly large deletions, can cause dominantly inherited Leri-Weill syndrome and the recessively inherited Langer mesomelic dysplasia. Studies have also found *SHOX* mutations in a small percentage of patients with idiopathic short stature. The Clinical Genetic Service receives referrals for genetic investigation of short stature. *SHOX* analysis has been provided to patients with short stature on a selective basis. *SHOX* mutations were detected in ten index patients, including nine females and one male. They have a phenotype that ranges from non-specific short stature, hypochondroplasia-like picture, to full-blown Leri-Weill syndrome with Madelung deformity. Four patients had *SHOX* deletions as a result of cytogenetically detectable abnormalities involving chromosome Xp [2 patients with t(X;Y) and 2 with Xp-]. Three patients had large deletions encompassing the entire *SHOX* gene. The other three patients had nonsense mutation, missense mutation and a 9-bp in-frame deletion, respectively.

P11-19:

CLONING AND SEQUENCING ANALYSIS OF ECHINOCCOCUS GRANULOSUS ANTIGEN CANDIDATE GENE

Zhao wei

Medical Molecular Biological Lab of Ningxia Medical College

Screening candidate antigen genes to prevent *Echinococcus granulosus*. **Method:** Through bioinformatics technology to acquire some gene sequences of *Echinococcus granulosus*, and amplify several of these genes sequences by RT-PCR, then sequencing and comparison DNA of these genes with the published sequences of *Echinococcus granulouses* in Genbank. Retesting these gene sequences which have some disparation with published sequence in Genbank. **Result:** The amplified twelve genes of *Echinococcus garnulosus* (they were ferritin, Hsp70, EF-1, Eg10, GST, 14-3-3, FABP, mMDH, myophilin, P-29, ZW-5, CaBP), and through comparison five of these gene sequences were different with published sequences, registered the five different genes in Genbank, and acquired the number of registration (the numbers were DQ678103, DQ678104, DQ678102, DQ679473，EF077179). **Conclusion:** These disparation genes are specific antigen gene of strains of Chinese *Echinococcus granulosus*, and may become valuable vaccines to prevent *Eg.*

P11-20:

A NOVEL MUTATION IN THE *EXT1* GENE IN A CHINESE KINDRED WITH HEREDITARY MULTIPLE EXOSTOSIS

Wen Wen, Yang Luo, Lihua Cao, Yang Zhang, Xue Zhang

The Research Center for Medical Genomics, China Medical University, Shenyang, 110001, China

Introduction: Hereditary multiple exostoses (HME) is an autosomal dominant bone disorder characterized by growths of multiple exostoses, benign cartilage-capped bone tumors that grow outward from the metaphyses of long bones. Penetrance is 95%. Three genes are known to be associated with HME: *EXT1*, *EXT2* and *EXT3*, located on 8q24.11-q24.13, 11p11-p12 and 19p, respectively. However, the second mutational hit may arise in a related gene such as the *EXT*-like genes *(EXTL*) or other genes involved in the signaling cascade of chondrocyte proliferation. The *EXTL* gene family is related to *EXT1* and *EXT2* by sequence homology, and currently consists of three members: *EXTL1*, *EXTL2* and *EXTL3*, located on 1p36.1, 1p11-p12 and 8p12-p22. **Results:** A three-generation Chinese kindred in which HME affected 7 members, with an age range of 16–65 year, was investigated. These affected members had multiple exostoses that were located at the juxta-epiphyseal regions of long bones as well as at other sites. All affected individuals had “short stature”. Individual □-4 had abnormal bone remodeling, showing shortening and bowing of the bone with widened metaphyses. The disease-causing gene of the family was linked to the *EXT1* locus on chromosome 8. A mutation, 1897delC, was detected in *EXT1* which was cosegregated with the disease phenotype. Mismatch primer amplification and RFLP analysis suggested that this mutation was not detected in all unaffected family members and normal control. **Conclusion:** The novel 1897delC mutation of *EXT1*, which resulted in FS L633 is the disease-causing mutation in the Chinese kindred with HME.

P11-21:

GENOMIC REARRANGEMENT AT 10q24 CAUSING ECTRODACTYLY MALFORMATION IN TWO CHENESE HAN FAMILIES

Lihua Cao, Yang Luo, Wei Yang, Shusen Wang, Xue Zhang

The Research Center for Medical Genomics, China Medical University Shenyang, 110001, China

Ectrodactyly or split hand–split foot malformation (SHFM) is a human congenital limb malformation characterized by hypoplasia/aplasia of the central digital rays and variable fusion of the remaining digits. Our aim in this study was to identify the disease-causing genetic alteration of ectrodactyly in two Chinese Han SHFM families. Photographs and roentgenograms showed that 2 affected females in family 1 show a typical lobster-claw anomaly, and one of them has campulitropous right forearm, while the affected male is absent of digits and metacarpals; and all affected individuals are lack of all metatarsals and phalanges as well as some cubitales or cuboids in both feet. Another feature in all affected individuals is short stature and 2 affected individuals have seizures. In family 2, all affected individuals show absence of 3 radial fingers, 2 of them have a deep central cleft and central ray deficiency in the feet, and one has a fibular monodactyly. Most limb malformations described above are bilateral and consistent with the phenotype of typical SHFM. The potential haplotype shared by all affected individuals was detected in the markers from SHFM3 loci. Comparative analysis in the family 1 after qPCR indicated a duplication of 480 kb DNA segment at 10q24.3, containing five genes-*LBX1*, *BTRC*, *POLL*, *DPCD* and *FBXW4* and in the family 2 another duplication of 580 kb segment in the same region harbouring *TLX1* gene. Our work may help to better understand the mechanisms of limb development. Further study will be required to explore the mechanisms of ectrodactyly and the relationship between the ectrodactyly phenotype disparity and the extent of the DNA duplication.

P11-22:

THE PRELIMINARY EXPLORE OF AFLP IN GENETICS ANALYSIS OF *CANNABIS SATIVA L.*

Pei Li[2], Guo Jia[1], Peng Jianxiong[2], Zhai Hong[3], Zhang Guiqin[1], Jie Qin[3], Tu Zheng [2]

*[1]Chinese People's Public Security University, Beijing,*100038*; [2]Institute of Forensic Science Ministry of Public Security P.R.C, Beijing,*100038*; [3]China Agricultural University, Beijing,*100094

The AFLP was used to analyze the genetic diversity of 12 varieties of *Cannabis sativa L.* and we selected five primer combinations which have good diversity from 55 primer combinations. The result showed that 285 AFLP bands were amplified using the five primer combinations, among which 99 bands were polymorphic and 10 bands were special, and 47～76 bands were amplified in each lane. In addition, this study demonstrates that AFLP fingerprinting has high sensitivity, stabilization and repetition, and the AFLP has much resolving power in the diversity study of *Cannabis sativa L. So this preliminary explore* will *establish a well foundation in the deeper research of* genetic diversity of *Cannabis sativa L.*

P11-23:

THE *STE17* GENE IS CODING FOR α-D-GLUCOSE-1-PHOSPHATE CYTIDYLYTRANSFERASE FUNCTIONAL IN EBOSIN BIOSYNTHESIS IN *STREPTOMYCES* SP. 139

Qi Xiaoqiang

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College

Ebosin, a novel exopolysaccharide produced by *Streptomyces* sp. 139 has medicinal activity and its biosynthesis gene cluster (*ste*) has been identified. In this study, the *ste17* gene was expressed in *E.coli* BL21, and the recombinant protein was purified. With CTP and α-D-glucose-1-phosphate as substrates the recombinant Ste17 protein was found capable of catalyzing the production of CDP-D-glucose and pyrophosphate demonstrating its identity as an α-D-glucose-1-phosphate-cytidylytransferase. To investigate the function of *ste17* in Ebosin biosynthesis, the gene was disrupted with a double crossover via homologous recombination. The monosaccharide composition of EPS-m produced by the mutant *Streptomyces* sp. 139 (*ste17*) was found significantly changed in comparison with that of Ebosin with glucose becoming undetectable. This gene knockout also negatively affected the antagonist activity for IL-1R of EPS-m. These results indicate that the *ste17* gene is involving in formation of glucose nucleotide as glucose precursors in Ebosin biosynthesis.

P11-24:

THE CLONE OF PNP GENE AND THE CONSTRUCTION OF ITS EXPRESSION VECTOR

Shi Xiaoyu, Rao Zechang, Li Wenlin, Zhao Lin

Nanchang University, Nanchang 330006, China

Objective: To construct and identify the recombinant vector pMSCV-PNP (purine nucleoside phosphorylase) which can express in eukaryote cells and will provide the basis for gene therapy. **Methods:** PNP were amplified from *E.coli* K12 bacteria by polymerase chain reaction (PCR). To construct the recombinant vector pMSCV-PNP by means of T4-DNA ligase. We transformed the competent bacteria with the plasmids pVSV-G, pGAG-POL, pMSCV and pMSCV-PNP. The plasmids cut by these restriction endonucleases were examined using agar electrophoresis. The recombinant retrovirus containing PNP target gene and the backbone recombinant retrovirus were packaged by 293 cells. **Results:** Ampicillin resistant tranformed bacteria colonies growth: After transformation with pMSCV, pMSCV-PNP, pGAG-POL and pVSV-G, about 100 colonies were observed on the plate containing ampicillin, and the colonies were not found in the negative control groups. The normal size of the fragments from the plasmids cut by endonucleases: The results of restriction endonuclease analysis and agar electrophoresis indicated that size of plasmids pMSCV, pMSCV-PNP, pGAG-POL and pVSV-G was normal. The recombinant retrovirus packaged by 293 cells: Green fluorescent was detected in 293 cells after transfection of 24 hours. **Conclusion:** PNP can be successfully cloned and inserted into eukaryote-expression vector. The newly constructed vector may serve as the potential tool to conduct further comprehensive experiments in future on PNP function and on gene therapy.

P11-25:

CLONING AND EXPRESSION OF HSP24 GENE FROM *TRICHODERMA HARZIANUM*

Bo Sun, Guangyou Wang, Dandan Wang

Department of Neurobiology, Harbin Medical University

Our research interests include the gene engineering and bio-information. Small heat shock proteins (shsps) are stress-inducible molecular chaperones by environmental. Such proteins are produced in most organisms. To study the defense molecular mechanism of sHSPs in *Trichoderma harzianum,* heat shock protein 24 (hsp24) was cloned from *T. harzianum* and expressed in *Escherichia coli*. Recombinant plasmid pYES2-HSP24 and vector pET28a were purified and cut by restriction enzyme *Eco*R□and *Xho*□. The target fragment was then connected with vector pET28a by ligase T4. Then expressing recombinant plasmid pET28-HSP24 was characterized by restriction enzyme digestion and sequencing. The expressing recombinant plasmid pET28-HSP24 was obtained. Meanwhile, it was demonstrated that the expressing fragment was HSP24 sequence indeed by aligning with GenBank (AY955084). The fusion protein is expressed in *E. coli* BL21 (DE3). With the induction by IPTG, the target protein was expressed in *E. coli* BL21 (DE3). An expression vector containing the open reading frame of the HSP24 gene was expressed in *E. coli*. This study presents a thermal-stress survival model for cells using the *E. coli* expression system for which *Trichoderma harzianum* HSP24, a recombinant protein, is inducible. Results show that *E. coli* cells overexpressing *T. harzianum* HSP24 have enhanced tolerance to 50□ thermal stress.

P11-26:

APPLICATION OF PERIPHERAL BLOOD LYMPHOCYTE TELOMERE LENGTH AS THE PREDICTOR FOR SIDE-EFFECTS OF CHEMOTHERAPY

Nelson LS Tang[1], Eddie WC Suen[2], Wei Huang[1], Frankie KF Mo[3], Winnie Yeo[3]

[1]Department of Chemical Pathology, [2]Department of Medicine & Therapeutics, [3]Department of Clinical Oncology, Faculty of Medicine, The Chinese University of Hong Kong

Adjuvant chemotherapy is a main stage treatment in many breast cancer patients. However, such treatment is associated with toxicity leading to side effects including symptoms of vomiting and pancytopenia. Leucopenia is an important side effect caused by toxicity of chemotherapy to the bone marrow. Telomere length shortened in the cell replication process, thus it may act as a predictor of side-effects that caused by the cell deaths. In our study, we would like to investigate if the treatment of breast cancer by chemotherapy might affect telomere length of the peripheral blood lymphocytes (PBLs). 32 breast cancer patients were recruited by the Prince of Wales Hospital in Hong Kong. Their telomere lengths were measured before and after treatments with an interval of about 2 months. The mean (SD) telomere length of the first measurement was 10.84kb (1.86kb) while that of the second measurement was 10.70kb (1.57kb). However, no significant difference of these telomere lengths was found (p=0.61). Within the 32 patients, 20 developed leucopenia of grade 3 or above while the remaining 12 patients did not. The mean 2-month changes of telomere length of the patients who developed leucopenia (grade3 or above) was -0.32kb while that of the other group was -0.04kb, but the difference was insignificant (p=0.64). Doing the same calculation, no significant difference could also be found for the patients with severe leucopenia (grade 4) and the remaining patients (p=0.28). In conclusion, chemotherapy does not alter the PBLs telomere length significantly. Telomere length may not predict the side-effects of chemotherapy accurately. This project is supported by the Hong Kong Anticancer Society.

P11-27:

GENE MUTATION TYPE ANALYSIS OF GLUCOSE-6-PHOSPHATE DEHYDROGENATE DEFICIENCY IN HAINAN NEWBORN INFANTS

Wang Jie, Huang Ci-dan, Zhao Zhen-dong, Yang Chun

Hainan Screening Centre for Neonatal Diseases, Hainan, Haikou, 570206

Objectives: To elucidate the molecular basis of G6PD deficiency in Hainan newborn infants through the gene detection and analysis of those who were diagnosed as G6PD deficiency infants by neonatal diseases screening. **Methods:** Fluorescence spot test and G6PD/6PGD rate test detecting of G6PD, analysis of amplified DNA sequences, PCR combined with oligonucleotide probes, PCR restriction fragment length polymorphism, single strand conformation polymorphism analysis technique were used to screen the mutations. **Results:** Of the 51 newborn infants of G6PD deficiency, 16 with G1 376T (31.37%), 11 with G1 388A (21.57%), 4 with A95G (7.84%), 2 with G392T (3.92%), 2 with G871A (3.92%), 1 with A835T (1.96%) and 1 with T517C (1.96%) were found. And yet 14 with no genotyping. **Conclusions:** The gene mutation type of G6PD deficiency in Hainan newborn infants is similar to the most common mutation type of G6PD deficiency in other areas. We do not find A835G-G6PD of Haikou type.

P11-28:

PREPARATION AND APPLICATION OF SCFV AGAINST BIOTOXIN

Shihua Wang, Feng Zhang

College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

Biotoxins are biogenic chemical substances displaying plentiful diversities that are up to several thousand kinds. In this study, we used phage display to obtain scFv (single chain fragment variable) against biotxin. The scFv DNA was ligated into a phagemid vector and the ligated product was then transformed into *E. coli* to yield recombinant phages after infection with helper phage. After of panning with biotoxin, the phage clones displaying scFv fragments of the antibody were selected by ELISA. Therefore, scFv antibody against biotoxin was prepared to study the possibility of plant resistance improvement through the expression of biotoxin scFv in plant.

P11-29:

CHARACTERIZATION OF TWO SOIL METAGENOME-DERIVED LIPASE WITH HIGH SPECIFICITY FOR P-NITROPHENYL PALMITATE

Ping Wei, Wen-Gang Song, Gang-Ping Hao

Department of Basic Medicine, Taishan Medical University, Taian 271000, Shandong Province

Two novel genes (pwtsB & pwtsC) encoding lipase were isolated by functional screening from soil metagenomic library. Sequence analysis revealed that pwtsB consisted of 906 bp encoding a protein of 301 amino acids with a molecular mass of 33 kDa and pwtsC consisted of 972 bp encoding a protein of 323 amino acids with a molecular mass of 35 kDa. Furthermore, both genes were cloned and expressed in Escherichia coli BL21 (DE3) using pET expression system. The expressed recombinant enzymes were purified by Ni-nitrilotriacetic acid affinity chromatography and characterized using spectrophotometric with different p-nitrophenyl esters. Result showed that PWTSB displayed a high degree of activity and stability at 20°C with an optimal pH around 8.0. PWTSC displayed a high degree of activity and stability at 40°C with an optimal pH of around 7.0. p-nitrophenyl palmitate was identified as the best substrate of PWTSB and PWTSC in present study. They had specific activity of 150 U/mg and 166 U/mg toward pNPP at 30°C, which is about 20-fold higher than that toward p-nitrophenyl butyrate (C4) and Caprylate (C8). Our data indicated that PWTSB and PWTSC were cold adapt lipase and thermostable lipase to long-chain p-nitrophenyl esters, respectively.

P11-30:

TOLL-LIKE RECEPTOR 3-INDUCED SUPPRESSION VIA BINDING OF SUPPRESSOR OF CYTOKINE SIGNALING 3 TO TYROSINE KINASE 2

Bin Zeng, Haijie Li, Yu Liu, Zhuohan Zhang, Yuan Zhang, Rongcun Yang

Department of Immunology, Nankai University School of Medicine, Nankai University, China, 300071

The Suppressor of cytokine signaling (SOCS) family of negative regulatory proteins are upregulated in response to several cytokines and pathogen-associated molecular patterns (PAMPs), and suppress cellular signaling responses by binding receptor phosphotyrosine residues. Exposure of bone marrow-derived dendritic cells (BMDCs) to 1D8 cells, a murine model of ovarian carcinoma, suppresses their ability to express CD40 and stimulate antigen specific responses in response to PAMPs, and in particular to polyI:C with the upregulated SOCS3 transcript and protein levels. The ectopic expression of SOCS3 in both the macrophage cell line RAW264.7 and BMDCs decreased signaling in response to both polyI:C and IFNα. Further, knockdown of SOCS3 transcripts significantly enhanced the responses of RAW264.7 and BMDCs to both polyI:C and IFNα. Immunoprecipitation and pull-down studies demonstrate that SOCS3 binds to the IFNα receptor TYK2. Since polyI:C triggers autocrine IFNα signaling, binding of SOCS3 to TYK2 may thereby suppress the activation of BMDCs by polyI:C and IFNα. Thus, elevated levels of SOCS3 in tumor-associated DCs may potentially resist the signals induced by TLR3 ligands and type I interferon to decrease DC activation via binding with IFNα receptor TyK2.

P11-31:

ANKMY2 PHYSICALLY INTERACT WITH FKBP8 AND SUPPRESS NFAT PATHWAY

Yin Zhaochu

Family Planning Institute of Hunan Province, 49 Middle Furong Road, Changsha, Hunan, P. R. China

We have cloned human Ankmy2 full length cDNA from human heart cDNA library, Northern blotting showed that Ankmy2 was expressed in heart, skeletal muscle, brain, liver, pancreas, spleen, kidney and testis at varied level. A GFP tagged Ankmy2 fusion protein was detected to localize in cytoplasm. Ankmy2 encode a 441 amino acid residue protein which contains three tandem ankyrin repeats in the amino terminal and one MYND domain in the carboxyl terminal, the ankyrin repeats mediate protein-protein interactions and are among the most common structural motifs in known proteins, The MYND domain is a conserved zinc binding domain and is also shown to mediate protein-protein interactions, we identified FKBP8 as an interaction partner of Ankmy2 through yeast two hybridization screening, this interaction was further confirmed by mammalian two hybridization experiment and coimmunoprecipitation. Transient expression of Ankmy2 in COS7 cells significantly decrease luciferase activity of NFAT reporter construction. FKBP8 can bind and stabilize Bcl2 to prevent cell apoptosis. These results suggest Ankmy2 may have a role in cell apoptosis and transcription regulation.

P11-32:

CLONING AND CHARACTERIZATION OF A NOVEL HUMAN GENE AC3-33 THAT INHIBITS AP-1 ACTIVITY

Xiujun Zhang

North China Coal Medical College, Jianshe Nan Road No.57, Tangshan 063000, P.R. China

The transcription factor AP-1 plays a role in the expression of many genes involved in cellular proliferation and cell cycle progression. AP-1 activity is increased in multiple human tumor types. Inhibitors of AP-1 have been shown to block tumor promotion, transformation, progression and invasion. AP-1 is composed of a mixture of heterodimeric protein complexes derived from the Fos and Jun families, including c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. AP-1 heterodimers bind to DNA on a serum response element with the 5´-TGA(C/G)TCA-3´ sequence. Here we report the cloning and characterization of a gene, named as AC3-33 (GenBank: c30rf33, Accession No. FLJ31139), which encodes a 251 amino acid protein. The AC3-33 gene consists of six exons, and is mapped to 3q25.31 by searching the UCSC genomic database. We successful constructed the ORF of AC3-33 into the mammalian expression vector pcDNA3.1B. 293T cells were transiently transfected with AC3-33-GFP expression vectors, and microscopy analysis of AC3-33-GFP fusion protein (green) was performed after 24h transfection. The result shows AC3-33 was localized to the cytoplasm. Northern blot analysis confirmed that AC3-33 mRNA expressed in many adult human tissues, such as brain, heart, testis, muscle and lung. RT-PCR analysis revealed that AC3-33 highly expressed in a variety of normal tissues, including muscle, brain, lung, was expressed at low levels in placenta and heart. AC3-33 mRNA was also detected in various cell lines by RT-PCR, expression levels were significantly higher in Hela, 293T, HT-29 and H1299 than that in Lncap. By dual luciferase reporter assay, AC3-33 was found to be able to inhibit transcriptional activities of AP-1 (PMA+Ionomycin). Furthermore, we confirmed the suppression effect of AC3-33 by using electrophoretic mobility shift assay (EMSA). Our findings suggest that AC3-33 is an inhibitor of AP-1 activation and that this protein may provide an alternative regulatory mechanism for AP-1 mediated transcription.

P12-1:

THE GENE EXPRESSION PROFILING IN MYELODYSPLASTIC SYNDROME AND ITS POSSIBLE CLINICAL USAGE

Xiaofei Qi, Jun Qian, Dandan Liu, Jiannong Cen, Hongjie Shen, Yuanyuan Wang, Jun He, Zixing Chen

Leukemia Research Division, Jiangsu Institute of Hematology, First Affiliated Hospital, Soochow University 215006 Suzhou

The myelodysplastic syndrome (MDS) is a heterogeneous clonal malignant myeloid disorders characterized by refractory cytopenia, dysplastic cellular morphology and a propensity of transformation towards AML. The clinical subtypes of MDS consisting of RA, RAS, RAEB, RAEBt represent the dynamic process of transformation from normal hematopoietic cells to leukemia cells. The diagnosis of MDS is currently based on morphology of BM cells and the prognosis scored by IPSS is most closely depending on the percentage of marrow myeloblasts and the presence of clonal cytogenetic abnormalities with a poorly understood molecular pathogenesis. We have applied Affymetrix microarray to analyze the gene expression profile of CD34+ cells from MDS patients in a hope of finding novel genes related to the pathogenesis of MDS and provide possible diagnostic biomarkers. The gene expression profiles of CD34+ cells from 8 MDS patients (2 RAEBt, 2 RAEB, 2RA, 1RAS, 1CAA) and normal controls hybridized to 10 microarrays containing18404 genes revealed a number of candidate genes which demonstrated differentially expression pattern between MDS and normal contols. Among them, up-regulated expression of Dlk-1 in MDS compared with AML and normal, down-regulation of PACAP in MDS in comparison with normal and a significant higher expression of RAP1Gap in MDS compared with AML, were all confirmed by qRT-PCR, respectively, in expanded cohorts of MDS patient. These results may have possible clinical usage in terms of distinguishing MDS-RA from chronic aplastic anemia (CAA) and predicting the risk of transformation from MDS-RAEB to AML.

P12-2:

REVIEW ON PHARMACOGENOMIC RESEARCH: EXPERIENCE OF A LOCAL IRB COMMITTEE AND PROFESSIONALS FROM SOUTHERN TAIWAN

Shio-Jean Lin, Meei-Ren Wang, Hwei-Ling Lee

Department of Pediatrics, National Cheng Kung University Hospital, 138, Sheng Li Road, Tainan, Taiwan

With the progress of clinical research in Taiwan, there are policy guidelines and regulations developed for protection of human research subjects. Institution Review Boards are performing in the role of gate keeper for clinical research. The study reported here aimed to explore IRB members' opinions and committee's operation on reviewing priorities for pharmacogenomics and genetic related research projects. From 2006 to 2007, among 766 clinical research projects reviewed by IRB of this teaching hospital in southern Taiwan, there was 6 pharmacogenomics research project and 56 genetic related research projects. We classify these projects as cancer genetics, susceptibility and gene therapy researches. We analyzed the approval process，timeline and reviewers' opinions in these cases. We found that the average time for approval was 67.8 days, the maximal is 166 days and the minimal is 15 days. About reviewers' opinions, there were several suggestions had been addressed repeatedly, including the format of informed consent and disposition of samples after research. They concerned about medical terms or obscure wording used in informed consent; in addition, there was no illustration about how to deal with the sample after research. About potential risk of clinical trila, PIs were worried that who would take responsibility for compensation. And for the general, leakage of genetic information was the most concerned.

P12-3:

HTRA1 POLYMORPHISMS IN EXUDATIVE AGE-RELATED MACULAR DEGENERATION

P OS Tam, TK Ng, DTL Liu, WM Chan, SWY Chiang, LJ Chen, DSC Lam, CP Pang

Department of Ophthalmology & Visual Sciences, The Chinese University of Hong Kong. 3/F Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong

Mapping the genes for age-related macular degeneration (AMD) had not been successful until recent genome-wide association studies revealed Tyr402His in *CFH* and rs11200638 in *HTRA1* as AMD-susceptible genetic variants. In this study, we aim to identify other critical factors in *HTRA1* that is associated with exudative AMD. The promoter, splice regions and coding exons of *HTRA1* were sequenced in 163 exudative AMD patients and 183 sex and age-matched control subjects. Also documented were the *CFH* genotype and smoking status. We found 4 significant SNPs in the promoter and the first exon of *HTRA1*: rs11200638 (-625G>A), rs2672598 (-487T>C), rs1049331 (102C>T, Ala34Ala) and rs2293870 (108G>T, Gly36Gly) with respective p-values = $1.7x10^{-14}$, $3.0x10^{-10}$, $3.7x10^{-12}$ and $3.7x10^{-12}$. Among them, rs11200638 is the most significant associated SNP with a high OR of 7.6 (95%CI: 3.94-14.51). One risk haplotype block across the promoter and exon 1, ACCTT, significantly predisposes to AMD (p= $6.68x10^{-14}$). Significant independent additive effects were identified in either model with smoking and rs800292 (184G>A, Val62Ile) of *CFH*. The combined OR for disease of smoking and rs11200638 (*HTRA1*) caused a 15.7 fold increased risk whereas that of combined rs800292 and rs11200638 showed a 23.3 fold increased risk. An extremely high population attributable risk (PAR) of 78% was also found. A high impact of the additive effect of *CFH* and *HTRA1* in the development of exudative AMD was shown in our study. HTRA1-smoking additive effect found in this study further suggests the importance of this environmental risk factor in AMD.

P12-4:

HAPLOTYPE ANALYSIS OF *GLI1* GENE WITH CONGENITAL HEART DISEASE IN 12Q13

Qiu Guangrong, Xin Na, Gong Liguo, Xu Xiao Yan, Liu Hongbo, Sun Kailai

Department of Medical Genetics, China Medical University, Shenyang, 110001, P.R.China

Objective In the candidate region 12q13 of simple Congenital Heart Disease (CHD),we chose four single nucleotide polymorphisms (SNPs) in *GLI1* gene and investigated individual SNP distribution and haplotype analysis in simple CHD patients and normal controls, in order to identify whether *GLI1* gene was the candidate for CHD or not. All subjects were from Northeast Chinese Han. **Methods:** We analyzed genotypes of 4 SNPs, which were C9455A, A10691G, G11388A, and G11888C, in 180 simple CHD patients and 200 normal controls by RFLP and DHPLC. Statistic analysis included Hardy-Weinberg equilibrium Assay, Association study at individual SNP, Linkage Disequilibrium Test, and Haplotype analysis. **Results:** There was no polymorphism at A10691G in the Northeast Chinese Han. The distribution of allele frequency and genotype frequency at G11888C which was located in the coding-region of *GLI1* gene, had significant difference between two groups ($P<0.005$). Linkage disequlibrium Test showed that there existed linkage disequilibrium among C9455A, G11388A and G11888C. Haplotype analysis showed that the distribution of haplotype had significant difference between two groups ($P<0.0001$). C9455/G11388/G11888 and C9455/A11388/G11888 were the common haplotypes in the population, but C9455/G11388/G11888 were much higher in CHD groups than that in normal controls ($P<0.05$). **Conclusion:** Together with our results in family-based association and Haplotype analysis, G11888C located in the coding-region of GLI1 gene was associated with simple CHD, person with G allele at G11888C has much more risk with simple CHD. C9455/G11388/G11888 might be linked with the susceptibility gene of simple CHD.

P12-5:

DETECTION OF THE COMMON CHROMOSOMAL ABNORMALITIES USING ARRY-BASED MLPA APPROACH

Dawen Zhou, Yihua Guan, Miao Xu, Jingbin Yan, Meijue Chen, Ying Huang, Zhaorui Ren

Shanghai Institute of Medical Genetics, Shanghai Children's Hospital, Shanghai Jiao Tong University

Aneuploidies are the most frequent chromosome abnormalities. Currently, the diagnosis of aneuploidies usually relies on karotyping analysis, which is labor-extensive, time-consuming and inefficient. Recently, we developed array-based MLPA method. It can detect more than 100 DNA sites or copy information within two days. We previously applied array-MLPA to detect DNA deletions and duplications in DMD gene, which proved the stability and reliability of the method. The present study is committed to apply array-MLPA to detect aneuploidies in clinical patients and to assess the accuracy in diagnose of aneuploidies as compared to karotyping analysis. Probes on several key gene sites on five chromosomes (13, 18, 21, X, Y) were designed, followed by detecting the copy number of each site to check the number of the chromosomes. A total of 200 clinical samples including 188 blood cell and 12 amnionic cell DNA samples were tested. 103 were checked out to be aneuploidis except 2 mosaicisms that were completely in accordance with karotyping analysis. The results demonstrated that the array-MLPA takes on a high rate for detecting number changes in chromosomes. As a method of gene-scanning, array-MLPA has the advantages in detecting micro-chromosome changes at submicroscopic level, and some small changes were tested in part of samples with a normal karotype. It also has a significant help for checking the accurate sites of some unknown markers. With great advantage in simple operation, high efficiency and reliability, array-MLPA has a practical value and potential in the application of molecular and prenantal diagnosis for aneuploidies.

P12-6:

ALTERED MICRORNA EXPRESSION ASSOCIATED WITH TRANSFORMATION OF ICE-6 CELL

Yan Wang, Xukai Wang, Bo Zhang

Department of Medical Genetics, Third Military Medical University, Chongqing 400038, China

Colon cancer is one of the most common cancers associated with considerable mortality and morbidity rates. Tumorigenesis of colon cancer is thought to be the consequence of gene mutation and disordered gene expression. Recent work indicated miRNA was involved in the development of colon caner. However, the detailed molecular mechanism underlying the development and progress of colon cancer remains unknown. To elucidate the miRNA associated with cancer biological pathways involved in transformation and tumorigenesis, we transformed normal IEC-6 cells to cancer cells by treatment with cancerogenic agent of MNNG and PMA. Cell proliferation was significantly increased as IEC-6 cells were treated with MNNG/PMA. Transformed IEC-6 cells were inoculated subcutaneously in nude mice and tumor xenografts were detected in all animals 4 weeks later. Then we investigated the altered miRNA at least a 2.0-fold expression increase or decrease of transformed IEC-6 cells by array hybridization (miRCURY Array v9.2, Exiqon). 13 miRNA were increased and 97 decreased. We were interested in increased miRNA and verification by real-time PCR showed miR-208 and 22* were up-regulated in transformed IEC-6 cells, which was coincident with that of microarray analysis. This implies that the data obtained from microarray analysis were reliable. Our results suggested miRNA with important biological roles were involved in transformation and tumorigenesis of IEC-6 cells, and this gave clues for further study to find out the molecular mechanism of development and progress of colon cancer.

P12-7:

WHOLE GENOME MICROARRAY EVALUATION OF GENOMIC IMBALANCE IN CHINESE PATIENTS WITH DEVELOPMENTAL DISORDERS

Yiping Shen[1]*, Xiaoli Chen[2]*, Hongyan Wang[3]*, Hong Shao[1], Shilin Li[3], Hong Fang[1], Xiaoming Shen[1], Li Jin[1,3]#, Ting Zhang[2]#, Bai-Lin Wu[1,3]#

[1]Children's Hospital Boston and Harvard Medical School, Harvard University, Boston MA, USA; [2]Capital Institute of Pediatrics, Capital Medical University, Beijing China; [3]Institutes of Biomedical Science and School of Life Science, Fudan University, Shanghai, China

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

Genomic imbalances/rearrangements are a common cause of developmental delay, mental retardation and autism spectrum disorders, which have been demonstrated by the studies with cohorts of the western countries. One type of such genomic imbalance appears recurrently with reciprocal microdeletion and duplication in different individuals involving the same genomic region and of the similar size, these events occur due to segmental duplication mediated non-allelic homologous recombination; another type of imbalance appears randomly and presumably occurs due to non-homologous end-joint recombination. The overlapping nature of regions involved in imbalance from individuals with similar clinical phenotype may eventually help to pinpoint critical regions/key genes responsible for such clinical phenotype.

In order to identify the genomic imbalances in Chinese patients with primary diagnosis of developmental disorders, we employed two most current high-resolution whole genome microarrays (Agilent 244K CGH array and Affymetrix SNP 6.0 array) to evaluate/compare the deletions and duplications in a pilot cohort consisting of 100 Chinese patients with mental retardation, global developmental delay and/or other cognitive impairment. We identified both types of genomic imbalances in the Chinese cohort: 16p11.2 deletion, 16p13.3 duplication and 17q12 duplication were examples of imbalance events that belonged to the first category (recurrent reciprocal imbalance), all of these microdeletion/duplication were recently recognized genomic disorders/syndromes; two unrelated cases with overlapping duplication involving 2p12 exemplified the second type of imbalance (randomly or de novo imbalance); the remaining findings belonged to the second category include 13q13.1q14.1 and 7q31.1 deletion. The high-resolution whole genome array also detected multiple imbalance

events in each patient sample but many events were benign copy number variants (CNV). Based on current database and literature, we identified clinically relevant genomic imbalances in about 13% of total cases.

In addition, we cross-validated the findings between the two DNA chip platforms and the results showed very high concordance rate. Our data demonstrated the utility of whole genome array (either array CGH or SNP genotyping array) for detecting genomic imbalance. The detection of recurrent genomic imbalance events among Chinese patient further confirmed shared etiologies among patients of different ethnicities. Since mental retardation is a broad clinical diagnosis of heterogeneous genetic etiologies, extensive clinical phenotype evaluation and further dissection of genomic rearrangement are of great importance towards better understanding of the genomic disorder and for eventual better patient care.

P14-1:

ANALYSIS OF GENE POLYMORPHISM OF THE ORIGIN REGION OF MITOCHONDRIAL DNA IN PILOTS

Cai Qing, Liu Hongjin, Zhan Ahi, Cheng Tao, Zhu Meicai

Department of Molecular Biology, Air Force General Hospital, Beijing, 100036

Research direction of author: Aviation medicine: 1.The molecular mechanism of brain damage induced by repeated high positive acceleration. 2. Study of related gene of high positive acceleration performance. **Research date:** From May 2007 to December 2007. **Objective:** To investigate the association between gene polymorphism of the origin region (D-loop) of mitochondrial DNA (mtDNA) and the performance of the pilots. **Methods:** The gene polymorphism of the D-loop of mtDNA was assessed in 86 pilots and 80 healthy control subjects by polymerase chain reaction and restricted fragment length polymorphism (PCR-RFLP). **Results:** There is a significant difference in distribution of the polymorphism of mtDNA D-loop between the pilots and healthy control subjects. **Conclusion:** The gene variation of mtDNA D-loop may contribute to the performance of the pilots.

P14-2:

INVESTIGATION OF GENEOME SNPS FOR KOREANS-CHINESE NATIONALITY IN YANBIAN OF CHINA

Zhang Zi-bo, Yang Kang-juan

Department of Cell Biology and Medical Genetics of Yanbian University College of Basic Medicine, Yanji 133000, Jilin, China

To search for hereditary feature of the Koreans-Chinese nationality genome SNPs in Yanbian of China, we used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), we detected four SNPs sites in Koreans-Chinese nationality: The polymorphism Pvu□ site of low density lipoprotein receptor (LDLR) gene, the polymorphism Pro12Ala site of peroxisome proliferator activated receptorγ2 (PPARγ2) gene, the polymorphism Msp□ site of alpha2(I) collagen gene (COL1A2), the CC chemokine receptor 5 (CCR5) Delta32 polymorphism (CCR5Δ32). At the same time, we studied dependability that gene polymorphism with disease. **Result and conclusion:** 1. The gene frequency of P^+ (presence of Pvu□ site) was 15% in 240 alleles of LDLR gene, the polymorphism Pvu□in intron 15 of LDLR gene was not visible associate with essential hypertension; 2. the allele mutation frequency is 4.1% for Pro12Ala site of PPARγ2 gene in 145 cases Korean-Chinese nationality and the Pro12Ala polymorphism of PPARγ2 gene is not visible associate with essential hypertension; 3. the gene frequency of P^+ (presence of Msp□ site) is 16.7% in 240 alleles of COL1A2 gene, the polymorphism site at COL1A2 gene was associated with Korean-Chinese nationality in Yanbian; 4. there was not the CCR5Δ32 genotype in observed 120 samples, the Koreans-Chinese of Yanbian might have a high susceptibility to HIV □ strain.

P14-3:

INTERFERON GAMMA RECEPTOR 1 GENE POLYMORPHISM IN TUBERCULOSIS PATIENTS FROM CHINA

Shiping Ding, Jianguo Wang, Kaijin Xu, Lanjuan Li

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, Zhejiang Province, China

Question of study: To examine the influence of the CA microsatellite polymorphisms of interferon gamma receptor 1 on patients with tuberculosis in the southeastern Chinese population. Materials and methods: Genomic DNA from patients with TB (n=155) and ethnically matched controls (n=89) were genotyped by short tandem repeat–PCR method. Results: The allele frequency of $(CA)_{25}$ was 1.70-fold higher among patients than that among controls (95% CI=1.07~2.70) (*P*=0.025). Compared with the non-$(CA)_{25}$/non-$(CA)_{25}$ reference group, the risk to tuberculosis of the carriers of $(CA)_{25}$/$(CA)_{25}$ genotypes were 6.46-fold (95% CI=1.40~29.74) (*P*=0.0017) higher. On the contrary, the allele frequency of $(CA)_{26}$ was 0.29-fold lower in cases than that in controls (95%CI=0.11~0.76) (*P*=0.012). Genotypes with $(CA)_{26}$ allele were at 0.35-fold (95% CI=0.13~0.98) (*P*=0.045) lower to the risk of tuberculosis, compared with that of the non-$(CA)_{26}$/non-$(CA)_{26}$ in the reference group. Conclusion: The above results indicated that the allele $(CA)_{25}$ appeared to be susceptible to tuberculosis while the allele $(CA)_{26}$ to be protective from tuberculosis. Our data also provided the CA repeat was a highly polymorphic marker and could be used for linkage and association analysis.

P14-4:

X-CHROMOSOMAL STRS POLYMORPHISM OF TWO ETHNIC GROUPS FROM SINKIANG (NORTHWEST CHINA)

Zhang Hong-Bo, Yang Li, Li Lin, Yu Bing, Lai Jiang-Hua

The Key Laboratory of National Ministry of Health for Forensic Sciences, College of Medicine, Xi'an Jiaotong University, Shaanxi 710061, China

Objective: To determine the genetic diversity of X-STR loci, and to evaluate the genetic structure of X chromosome in two ethnic groups (Kirghiz, Kazakh) from Sinkiang Uygur Autonomy Region of China (Northwest China). **Methods:** DXS6804, DXS6799 and DXS7132 loci were genotyped by multiplex PCR and their genetic parameters were analyzed by SPSS 13.0, Fstat, Powerstats and Phylip, respectively. **Results:** There were 20 alleles and 24 genotypes detected in 100 Kazakh unrelated persons. The gene frequencies ranged from 0.0063 to 0.6139, and the genotypes frequencies ranged from 0.0172 to 0.4310. There were 20 alleles and 26 genotypes detected in 100 Kirghiz unrelated persons. The gene frequencies ranged from 0.0067 to 0.5570, and the genotypes frequencies ranged from 0.0204 to 0.3265. The data of the 3 X-chromosome STRs in Kirghiz and Kazakh ethnic group of China are in accordance with Hardy–Weinberg equilibrium: The exact test yielded p-value from 0.1345 to 0.8313. Heterozygosity (H), the power of discrimination (PD) and the polymorphism information contain(PIC) were consistent with the forensic application. Cluster analysis and phylogenic trees demonstrated the genetic affinity between Kazakh and Kirghiz Populations. **Conclusion:** The genetic information demonstrates that the three loci were highly informative loci and are suitable for population genetics research and forensic application.

P14-5:

Y CHROMOSOME HAPLOTYPES REVEAL PATERNAL GENETIC STRUCTURE OF HAINAN ABORIGINES

Dongna Li,[1] Hui Li,[2] Caiying Ou,[1] Yan Lu,[2] Yuantian Sun,[1] Li Jin,[2]

[1]Department of Biology, Hainan Medical College, Haikou, Hainan, China; [2]MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China

At the southern entrance to East Asia, population migration has affected much of the chromosome variation of East Asians. To assess the original genetic structure at the southern entrance, variations of 405 Hainan Island aborigines from all the six populations were analyzed. The Y-haplogroup data indicate that 5 Hlai subgroups have a common ancestry,and the Hlai populations are quite different from the mainland mixed populations. The Y chromosome haplogroups O1a* and O2a* are dominant among Hainan aborigines Hlai subgroups. In addition, the frequency of the mainland dominant haplogroup O3 is quite low among these aborigines, indicating that they have lived rather isolated. Clustering analyses suggest that the Hainan aborigine Hlai has been segregated since about 20 thousand years ago, after two dominant haplogroups entered East Asia (31 to 36 thousand years ago). Our results suggest that the Y-SNP haplogroup patterns of the Hainan aborigines Hlai are closest to the original genetic structure of the early migrants at the entrance of East Asia, whose genetic characteristics could be used as important controls in many population evolution and genetic studies.

P14-6:

ASSEMENT OF ECHINOCOCCUS GRANULOSUS POLYMORPHISM IN QINGHAI PROVINCE, PEOPLE'S REPUBLIC OF CHINA

S.M. Ma[1,2], S. Maillard[1], H.L. Zhao[2], X. Huang[2], H. Wang[2], P.L. Geng[2], J.-M. Bart[1], R. Piarroux[1]

[1]Laboratory of Parasitology and Mycology, University of Franche-Comté, UFR SMP, 19 Rue Ambroise Paré, 25000 Besançon, France; [2]Institute of Parasitology, Medical College of Qinghai University, Qinghai Province, 16 KuLun Street, Xining City, 810001 China

Cystic Echinococcosis (CE), one of the world's most widespread parasitic diseases, is especially virulent in the Chinese province of Qinghai, located on the Tibetan Plateau. The *E. granulosus* sheep strain has already been reported in this focus. In order to improve our understanding of the role the parasite plays in the high prevalence observed in humans, we assessed the genetic polymorphism of 55 hydatid cysts (37 from humans) using three discriminative mitochondrial markers: *coxI, nadI* and *atp6.* A total of 1327bp were sequenced and 13 distinct genotypes (G1M1, G1M2, G1M3, G1M4, G1M5, G1M6, G1M7, G1M8, G1M9, G1M10, G1M11, G1M12 and G1M13) were identified which were all related to the common sheep G1 strain. 13 genotypes in our study were accepted by GenBank and registered under the GenBank Accession numbers (EU072106, EU072107, EU072108, EU072109, EU072110, EU072111, EU072112, EU072113, EU07214, EU072115, EU072116, EU072117 and EU072118). Six of these genotypes (G1M5, G1M6, G1M7, G1M8, G1M10 和 G1M12)have already been reported in China and other foci around the world. The remaining seven genotypes (G1M1, G1M2, G1M3, G1M4, G1M9, G1M12 and G1M13) were new variants of the strain. The parasite population which was studied in the present work did not differ significantly from those observed in other foci of CE. Environmental conditions and human behaviour could explain the high incidence of the parasitic disease, particularly in the Tibetan population living in the South of Qinghai, where most are livestock farmers. CE is a major public health problem in China and it is particularly serious in rural provinces.

P14-7:

GENETIC EPIDEMIOLOGY STUDY OF SYSTEMIC LUPUS ERYTHEMATOSUS IN A CHINESE POPULATION

Hai-Feng Pan, Xue-Hui Fang, Lian-Hong Li, Wen-Xian Li, Jin-Bao Feng, Hui Yuan and Dong-Qing Ye

Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei, Anhui, 230032, PR China

Objective: To explore the effect of genetic factors on the occurrence of Systemic lupus erythematosus (SLE) and analyze the general genetic pattern of SLE in a Chinese population. **Methods:** A case-control study of 166 SLE probands and 383 controls was performed to analyze the effect of genetic factor on the susceptibility to SLE. The heritability was estimated by Falconer's method. The genetic pattern was evaluated with Penrose's method. **Results:** 20 (12.05%) of 166 probands had one or more first-degree relatives suffered from SLE. Prevalence of SLE in first-degree relatives and second-degree relatives in proband group were 1.698% and 0.606% respectively, which were significantly higher than that in control group (0.1115%) (P<0.01). Furthermore, the odds ratio (OR) and its 95% confidence interval (95%CI) were 14.88 (4.22-62.70) and 5.31 (1.38-23.95) respectively. The order of prevalence was as follows: first-degree relatives >second-degree relatives>third-degree relatives>control relatives. A ratio of s/q approached $1/q^{1/2}$ with Penrose's method. Heritability of SLE was 78.8%±4.45% in the first-degree relatives, 58.8%±10.9% in the second-degree relatives, and 39.2%±32.0% in the third-degree relatives. The weighted mean heritability was 75.2%±4.12%. **Conclusion:** SLE has characteristics of polygenic disease. Genetic factor might play an important role in the susceptibility to SLE.

P14-8:

GENETIC POLYMORPHISM OF FXIIIB IN BEIJING HAN POPULATION

Ying Zhang[1], Li Pei[1], Jian Wang [2], Hua Deng[1], Chengtao Jiang[1], Xingchun Zhao[1]

[1]Institute of Forensic Science, Ministry of Public Security, Beijing 100038, China; [2]The Ministry of Public Security, Beijing 100741, China

The polymorphic of DNA and protein of coagulation blood factorXIII (FXIIIB) in Beijing Han population was investigated for the first time. There are 148 individual blood samples which were calculated the distribution of FXIIIB alleles frequency by Polymerase Chain Reaction. The results showed that a majority of samples contain 8, 9, 10 allele, their frequency are 0.0676, 0.2364 and 0.6892, which were in agreement with the expected numbers under the Hardy-Weinberg equilibrium. The research also includes the examination of FXIIIB STR loci on the stale blood and semen stain about 10 to 23 years ago. The same results compared with the blood sample proved that FXIIIB is useful in forensic work. Besides, isoelectrofocusing technique on polyacrylamide gels followed by immunoblotting was used to determine the phenotype of individuals in 259 samples, and 5 phenotypes of FXIIIB have been found. There are 3 alleles in all the samples. The frequency of FXIIIB*1, FXIIIB*2 and FXIIIB*3 are 0.3552, 0.0174 and 0.6236. All of them reached to the polymorphism's level. FXIIIB is a useful genetic marker for forensic individual and parentage identification.

P14-9:

THE MOLECULAR ANALYSIS OF SMN1 GENE IN CHINESE PATIENTS WITH SPINAL MUSCULAR ATROPHY

Song Fang, Long Mei-juan, Qu Yu-jing, Jin Yu-wei, Wang Hong

Department of Medical Genetics, Capital Institute of Pediatrics, Beijing, P. R. China 100020

SMA is an autosomal recessive disorder that results in symmetrical muscle weakness and wasting due to degeneration of the anterior horns of the spinal cord. The survival motor neuron (SMN) gene is found on chromosome 5q13. SMN1 is homozygously deleted in approximately 95% of patients worldwide. In order to elucidate the molecular basis of SMA in the China population, a total of 267 children patents with SMA were included in the study from 2003, Jun to 2006, Nov. The polymerase chain reaction restriction enzyme assay is used to detect the homozygous deletion in SMA patients. A dosage assay enabling the detection of SMN1 deletion heterozygotes was also developed. Results of the current study show that 81.3% (217/267) of SMA patients have homozygous deletions of SMN1 gene. The SMN1 heterozygous deletion was confirmed in 18.7% (33/50) of non-deletion SMA patients. The frequency and pattern of deletions in the Chinese children SMA patients are significantly different with observed in other international populations. Further gene characterization and subtle mutations within the SMN1 gene would need to be studied in order to define the molecular basis of SMA in the Chinese population.

P14-10:

GENETIC POLYMORPHISMS OF CYTOCHROME P450 ENZYMES 2C9 AND 2C19 IN A HEALTHY MONGOLIAN POPULATION OF CHINA

Xiulan Su, Zhenfang Yang, Hongwei Cui, Maolian Gong

Huhhte Tongdao North Street 1, Clinical Medicine Research Center of Inner Mongolia Medical College 010050

To explore the distribution characteristics of CYP2C9 and CYP2C19 gene polymorphisms for Mongolian population in China and provide the relevant data for studying the genotype of ethnic minority of the Inner Mongolian, using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) genotyping assays, the frequencies of functionally important variation of the cytochromes P_{450} (CYP) 2C9 and 2C19 were determine in a sample of 280 unrelated healthy volunteers derived from the area of xilinguole Meng in Inner Mongolia of China. The frequencies of the CYP2C9*1, *3 in the Mongolian population were 0.97 (95% confidence interval (CI) 0.94-0.99) and 0.04 (95% CI 0.00-0.17) respectively. CYP2C9*2 allele was not found in this study. The frequencies of CYP2C19*1, *2 and *3 alleles were 0.72(95% CI 0.62-0.80), 0.24 (95% CI, 0.16-0.32), and 0.04(95% CI, 0.02-0.06) respectively. Based on our study, the frequencies of CYP2C9*2, CYP2C9*3 allelic variants in Mongolian population of China are similar to those in other Asian population; the frequencies of CYP2C19*2, CYP2C19*3 allelic variants are higher compared with Caucasians.

P14-11:

X-CHROMOSOMAL STRS POLYMORPHISM OF TWO ETHNIC GROUPS FROM SINKIANG (NORTHWEST CHINA)

Yu Bing

The Key Laboratory of National Ministry of Health for Forensic Sciences, college of medicine, Xi'an Jiaotong University

Objective: To determine the genetic diversity of X-STR loci, and to evaluate the genetic structure of X chromosome in two ethnic groups (Kirghiz, Kazakh) from Sinkiang Uygur Autonomy Region of China (Northwest China). **Methods:** DXS6804, DXS6799 and DXS7132 loci were genotyped by multiplex PCR and their genetic parameters were analyzed by SPSS 13.0, Fstat, Powerstats and Phylip, respectively. **Results:** There were 20 alleles and 24 genotypes detected in 100 Kazakh unrelated persons. The gene frequencies ranged from 0.0063 to 0.6139, and the genotypes frequencies ranged from 0.0172 to 0.4310. There were 20 alleles and 26 genotypes detected in 100 Kirghiz unrelated persons. The gene frequencies ranged from 0.0067 to 0.5570, and the genotypes frequencies ranged from 0.0204 to 0.3265. The data of the 3 X-chromosome STRs in Kirghiz and Kazakh ethnic group of China are in accordance with Hardy–Weinberg equilibrium: The exact test yielded p-value from 0.1345 to 0.8313. Heterozygosity (H), the power of discrimination (PD) and the polymorphism information contain (PIC) was consistent with the forensic application. Cluster analysis and phylogenic trees demonstrated the genetic affinity between Kazakh and Kirghiz Populations. **Conclusion:** The genetic information demonstrates that the three loci were highly informative loci and are suitable for population genetics research and forensic application.

P14-12:

DETECTING GENOMIC COPY NUMBER VARIANTS IN DIFFERENT CHINESE ETHNIC GROUPS USING AFFYMETRIX SNP ARRAY 6.0

Shilin Li[1]*, Yiping Shen[2]*, Hong Shao[2], Hongyan Wang[1], Li Jin[1]#, Bai-Lin Wu[1,2]#

[1]Institutes of Biomedical Science and School of Life Science, Fudan University, Shanghai, China; [2]Children's Hospital Boston and Harvard Medical School, Harvard University, Boston MA, USA

** Equally contributed. # Co-corresponding authors.*

Genomic diversity between individual human beings is more extensive than previously realized. The existence of widespread genomic copy number variants (CNV), along with single nucleotide polymorphism (SNP), has important implications in understanding human diversity, evolution and disease susceptibility. Characterization of CNV and SNP in the DNA of representative health persons from Chinese ethnic groups is the first important effort toward larger genome-wide association studies in Chinese population. We have validated and implemented the most recent high-resolution Affymetrix SNP array 6.0 for simultaneous interrogation of CNV and SNP. Here we report the initial findings from 24 Han and 24 Tibetan Chinese healthy individuals. We detected and defined the fine structural genomic variation in Chinese individuals. We compared the CNV profiles between Han and Tibetan Chinese, identified both known and novel CNVs. The CNV detected in this cohort are compared with the data generated with the same array platform from 400 individuals of different ethnicities including 100 Asian, 100 African American, 100 Caucasian and 100 Mexican. Some novel CNVs are further confirmed by an independent method. Furthermore, the linkage disequilibrium (LD) between CNV and haplotype is exploited based on these datasets. We believe this is the first step towards understanding the unique structural genomic variation of Chinese individuals and the datasets form this foundation is powerful for more extensive case-control and family-based association studies, which can be conducted to correlate specific genetic variants with differential susceptibility to common diseases.

P15-1:

MULTIPLE GENE POLYMORPHISMS IN THE COMPLEMENT FACTOR H GENE ARE ASSOCIATED WITH EXUDATIVE AGE-RELATED MACULAR DEGENERATION

LJ Chen,[*] TK Ng,[*] DTL Liu, POS. Tam, WM Chan, K Liu, YJ Hu, SWY Chiang, DSC Lam, CP Pang

Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China

Purpose: Variants in the complement factor H gene (*CFH*) have been shown to be strongly associated with age-related macular degeneration (AMD). In our study, sequence alterations in *CFH* were investigated in 163 Chinese patients with exudative AMD and 155 unrelated Chinese control subjects. **Method:** All the 22 *CFH* exons, intron-exon boundaries and the promoter sequence were screened by polymerase chain reaction and DNA sequencing. **Result:** We identified 58 sequence changes, 42 of them were novel. Six SNPs with an allele frequency >30% were significantly associated with exudative AMD. SNP rs3753396 was novel, and the rest previously reported: rs3753394, rs551397, rs800292, rs2274700, and rs1329428. Two haplotype blocks were constructed. The TG haplotype for rs551397 and rs800292 was the major haplotype that conferred a significantly increased susceptibility for exudative AMD (P_{corr}=0.0001, OR=1.91, 95% CI: 1.36-2.68). **Conclusion:** Our findings enrich the evidence on the *CFH* gene as one of the AMD associated genes. There is a different distribution pattern of *CFH* variants in Chinese as compared to other populations. Individual SNP analysis and haplotype analysis revealed that the ancient alleles at the 5' end of *CFH* attribute to increased susceptibility of exudative AMD.

P15-2:

A PILOT STUDY ABOUT THE NON-SPECIFIC MENTAL RETARDATION AND COGNITIVE ABILITY CANDIDATE GENES

Fuchang Zhang, Xiaocai Gao, Kejin Zhang, Pingyuan Gong

College of Life Science, Institute of Population and Health, Northwest University Xi'an, China 710069

The research on the genes related to nonspecific mental retardation (NSMR) and cognitive ability has become a hotspot in cognitive nerve biology in recent years. To determine the relationship between NSMR of children and general cognitive ability, genetics markers was used to detect 8 NSMR genes (*GDL3*, *IL1RAPL1*, *ARX*, *FTSJ1, IL1RAPL1*, *IL1RAPL2* and *FTSJ1*) in X chromosome based on a case–control study or random-selected population study of Qinba Mountainous area children. The results show: (1 There are significant positive correlations between *GDL3*, *IL1RAPL1*, *ARX* and *FTSJ1* genes and NSMR of children, which suggests that some harmful mutations in these genes be causes underlying NSMR or general cognitive disability in this area. (2 The significant positive association of *IL1RAPL1*、*IL1RAPL2* and *FTSJ1* genes with intelligence or general cognitive ability with gender specific effects indicated that, the general cognitive ability of different genders is influenced by these functional polymorphisms with the genes.

P15-3:

STUDY OF THE GENETIC ASSOCIATION BETWEEN THE POLYMORPHISM OF CYTOSOLIC PLA2 FAMILY GENES AND SCHIZOPHRENIA

YU Ya-qin

Jilin University Research Center for Genomic Medicine, School of Public Health, Jilin University, Changchun 130021*, China*

Objective: To investigate the genetic association between the polymorphism of cytosolic phospholipase A2 (cPLA2) family genes and schizophrenia in the North Han Chinese. **Methods:** The method of PCR-based ligase detection reaction (PCR-LDR) was applied to genotype 21 single nucleotide polymorphisms (SNPs) of cPLA2 family genes among 201 pedigrees consisting of fathers, mothers and affected offsprings with schizophrenia. The pedigrees were collected in 2000-2006. Haplotype relative risk (HRR) test, transmission disequilibrium test (TDT), haplotype transmission analysis and multiple locus analysis were conducted to analyze the genotyping data. **Results:** The genotypic frequency of cPLA2 gene did not deviate from Hardy-Weinberg equilibrium in both case and control groups. HRR and TDT showed that there was significant difference for the frequencies of alleles of rs1549637 at PLA2G4C locus between the case and the control ($\chi 2 = 5.341$, $P = 0.021$; $\chi 2 = 5.633$, $P = 0.018$). Analysis for haplotype transmission showed that no haplotype systems was associated with schizophrenia ($P>0.05$). The COA and COG test showed a disease association for the rs2162886-rs1668589, rs891014-rs1668589 and rs2307279-rs7542180 combinations ($\chi^2 = 6.913$, $P = 0.032$; $\chi^2 = 8.393$, $P = 0.015$; $\chi^2 = 8.447$, $P = 0.038$). **Conclusions:** There is a genetic association between the PLA2G4C (rs1549637) locus and schizophrenia. There are many loci in the cPLA2 family genes, which associate with the schizophrenic clinical symptoms.

P15-4:

THE COMMON GENETIC ARCHITECTURE OF TYPE 2 DIABETES AND SCHIZOPHRENIA

Mao Sheng YANG

Laboratory of Disorder Genes, School of Public Health, Chongqing University of Medical Sciences

Type 2 diabetes mellitus is a heterogeneous and complex disease presumably with a multifactorial etiology comprising genetic and environmental factors. Schizophrenia is considered as a neurodevelopmental disorder of central nervous system, with high heritability and a likely complex genetic architecture. Much genetic evidences have accumulated in the past several decades for the two disorders but no gene has been unequivocally identified as containing risk variants for them. In this article we review the past and present literature on Pathological, Genetic linkage, and Epidemiological studies in the two disorders. We sought convergent evidence to support that there are common genes or chromosomal regions underlying the etiology for both Type 2 diabetes and Schizophrenia. Convergent evidence from these studies supports the current hypotheses that there are common genetic basis for Type 2 diabetes and Schizophrenia, and that genes involved in metabolism and neurodevelopment are especially important for future studies. Convergent evidence suggests the following nine chromosome regions 10q26, 18p11.3, 2q37, 6q23, 22q13, 12q24, 8p22, 1p31 and 1q23, are likely to contain sharing risk genes for the two disorders. Combining results from pathological studies, genes involved in energy metabolism and development located at the above regions should be prioritized for future genetic research.

P15-5:

REGULATION OF *nNOS* GENE TRANSCRIPTION BY p300-INDUCED NF-κB ACETYLATION

Yinghui Li, Miao Li, Chunyi Li, Yanyan Zhao

Department of Medical Genetics, China Medical University

Nitric oxide (NO) plays highly diversified biological roles in the nervous system. Among the three isoforms of NO synthase (NOS) (neuronal, nNOS; inducible, iNOS; and endothelial, eNOS), nNOS is predominant in the neuronal cells. The transcriptional regulation of *nNOS* gene is crucial for the nNOS-derived NO biosynthesis. Acetylation is a common epigenetic modification in regulating gene transcription. p300 has been demonstrated to be an important transcription coactivator that holds the activity of histone acetyltransferase. Nuclear factor (NF)-κB is a widely functioned transcription factor family subject to acetylation. In the present study, an NF-κB responsive element was identified within *nNOS* promoter by electrophoresis mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP). Results from coimmunoprecipitation suggested p300 may interact with NF-κB p65 and p50 subunits and ChIP assay indicated p300 may bind to the region of the identified NF-κB element within the *nNOS* promoter in human neuroblastoma SK-N-SH cells. Meanwhile, by transfection of p300 expression vector and immunoprecipitation combined with Western blot, p300 was demonstrated to directly acetylate p65 and p50 and their acetylation may further increase their bindings to *nNOS* promoter. Lastly, luciferase assay was carried out and showed p300-induced NF-κB p65 and p50 acetylation greatly increased the transcriptional activity of *nNOS* promoter. Taken together, our study provides a possible mechanism by which acetylation regulates *nNOS* gene transcription in the nervous system.

P15-6:

CYP4F2 WAS INVOLVED IN THE PATHOGENESIS OF HYPERTENSION

Hong Liu, Xiaoliang Liu, Yanyan Zhao

Department of Medical Genetics, China Medical University, Shenyang, 110001, China

Cytochrome P450 4F2 (CYP4F2) catalyzes the ω-hydroxylation of arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE), a natriuretic and vasoactive eicosanoid that participates in the development of hypertension. The relationship among *CYP4F2*, formation of renal 20-HETE, and hypertension is unknown. Here are reported an association of *CYP4F2* variants with urinary 20-HETE and hypertension in a Chinese population, and a *CYP4F2* transgenic mice model with CYP4F2 overexpression, increased 20-HETE and blood pressure. The 91T/C as a tagSNP was genotyped in a case-control study, and showed an association with hypertension after adjustment for risk factors such as age, gender and body mass index, which was confirmed in a family-based association study. This tagSNP was also associated with elevated 20-HETE. Functional study by electrophoretic mobility shift assay and reporter assay revealed that this tagSNP altered NF-κB binding affinity as well as transcriptional activity. To further investigate the function of CYP4F2 in the pathogenesis of hypertension, we generated a *CYP4F2* transgenic mice model. The *CYP4F2* transgenic mice showed overexpression of CYP4F2 in the kidney, and increased ω-hydroxylase activity of renal microsomes. In addition, transgenic mice also had significantly higher urinary 20-HETE levels and systolic blood pressure than the control. These results indicate that CYP4F2 plays a vital role in the development of hypertension through a prohypertensive action of 20-HETE.

P15-7:

DENTIFICATION OF UTS2 AND TNFRSF9 GENETIC CANDIDATE SUSCEPTIBILITY GENES TO TYPE 2 DIABETES IN NORTHERN CHINESE

Sun Hong[1], Wang Bin-you[1], Sun Liang[2], Yang Ze[2]

[1]Public Health College, Harbin Medical University; [2]Beijing Institute of Geriatrics, Minitry of Health, Beijing Hospital

Type 2 Diabetes is a complex syndromes caused by multiple heterogeneous genetic factors and environmental factors, strong genetic susceptibility are supposed to be involved. Therefore, we carried out a survey and scanning of 120 type 2 diabetes pedigree families, targeted Urotension and TNFRSF9 as candidates in order to study the associations with type 2 diabetes in Han nationality in northern Chinese. Using Case-Control design and PCR-RFLP techniques, we try to investigate the association between these genes and type 2 diabetes in this population. About 210 Type 2 Diabetes patients and 319 normal controls from Harbin, Mudanjiang, Daqing and Suihua City in Heilongjiang Province were involved. In our study, we found SNP rs2071984 C→T polymorphism between Urotension □gene and TNFRSF9 gene and the SNPrs161810 C→T polymorphism in the Urotension □gene were showed significant relative with Type 2 Diabetes Mellitus in northern China. The degree of correlation between the polymorphisms on these genes and Type 2 Diabetes Mellitus shows certain variance under different backgrounds. Genotype CC and allele C had positive relation with the risk of Type 2 Diabetes Mellitus, and genotype TT and allele T had negative relation with the risk of Type 2 Diabetes Mellitus. SNPrs228648 G→A polymorphism in Urotension □gene was not associated with T2DM. (Supported by the National Natural Fund of China：C030107-30671802)

P15-8:

ROLE OF CHD7 GENE IN THE ETIOLOGY OF FAMILIAL AND SPORADIC IDIOPATHIC SCOLIOSIS IN CHINESE ADOLESCENTS

Nelson LS Tang[1], Hiu-Yan Yeung[2], Rachel Kwok[2], Vivian WY Hung[2], Kwong-Man Lee[3], Tze-Ping Lam[2], Bobby KW Ng[2], Yong Qiu[4], Jack CY Cheng[2]

[1]Laboratory for Genetics of Disease Susceptibility, The Chinese University of Hong Kong; [2]Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong; [3]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong; [4]Spine Unit, Department of Orthopaedics, Nanjing University, Nanjing, China

Introduction: CHD7 gene, causing a familial syndrome of CHARGE, was recently associated with familial form of idiopathic scoliosis (IS). The haplotype and alleles showing disease predisposition were also common polymorphisms found in the population. Hence, these high-risk alleles in CHD7 gene may play a role in the genetic predisposition of sporadic IS as well as familial. This study examines the common CHD7 genetic polymorphisms predispose to familial and sporadic IS in the Chinese population. **Methods:** Five hundred and sixty-two Chinese females with IS (Cobb's angle>20) were recruited from a region-wide screening program and had been followed up till skeletal maturity. Four hundred fifty-three patients were sporadic and the other 109 had at least one first degree affected relative were classified as familial. Two hundred fifty-two healthy Chinese female adolescents were recruited as control. The population genetic variation in CHD7 gene was represented in the HapMap data. Tagging SNPs were identified by a Spectral Decomposition Analysis (Factor Analysis) using genetic r2 as the correlation parameter. Four tagSNPs were found accounting for 81.25% of the haplotypes. Two additional tagSNPs were chosen from the original report for replication. Genotyping was performed by PCR-RFLP. **Results:** Among the sporadic cases, SNP rs1483207 showed a borderline association at genotypic analysis (p=0.03) but it was not significant at allelic analysis (p=0.6). The spurious association was caused by a slight departure from Hardy-Weinberg equilibrium in the sporadic case sample. However, there was no association between these tagSNPs and familial IS. **Conclusion:** There was no association of the tagSNPs in CHD7 gene with Chinese sporadic and familial IS. There was a high level of linkage disequilibrium within this gene and most of the common haplotypes are shared between Chinese and Caucasian. Thus, this

tagSNP approach would detect any disease association with a common haplotype. The absence of association with CHD7 gene in this study indicated that common variants in CHD7 might play only a minor role in genetic predisposition to sporadic IS, at least in the Chinese population. However, we cannot exclude the presence of family-specific (private and rare) mutations in CHD7 causing familial IS by this approach.

P15-9:

ASSOCIATION OF STEROL REGULATORY ELEMENT BINDING PROTEINS-1C GENE POLYMORPHISM WITH TYPE 2 DIABTETS MELLITUS, INSULIN RESISTANCE AND BLOOD LIPID LEVELS IN CHINESE POPULATION

Xie Xiao-dong

The Institute of Genetics, School of Basic Medicine, Lanzhou University, Lanzhou,730000, Gansu Province

Background/Aims: The sterol regulatory element–binding protein (SREBP)-1c gene has been identified as a susceptibility gene in metabolic diseases such as type 2 diabetes mellitus (T2DM)，obesity, dyslipidemia and insulin resistance. Previous studies suggest that the single nucleotide polymorphism SNP17 (rs2297508, exon18c, G952G) of SREBP-1c gene and a common SREBP-1c SNP6 (rs11868035) are associated with an increased risk of T2DM. The present study aimed to confirm the previously reported association in a Chinese population and to examine the two SREBP-1c SNPs for their associations with diabetes-related quantitative traits such as insulin resistance and blood lipid. **Methods:** We genotyped two SREBP-1c SNPs in a case-control study (n=234) from Chinese, including 112 patients with T2DM and 122 healthy controls, using polymerase chain reaction-denaturing high-performance liquid chromatography (PCR-DHPLC) and tested for association with type 2 diabetes, insulin resistance and blood lipid , respectively. Genotype and allele distributions and haplotype construction were analyzed. **Results:** The genotype and allele distributions of rs2297508 and rs11868035 polymorphisms were significantly different in type 2 diabetic patients compared to controls (P=0.009 and P=0.036; P=0.001 and P=0.008, respectively). Haplotype analyses showed significant association with diabetes risk and confirmed the results of the single SNP analyses. In comparison with controls, cases had significantly higher levels of WHR, SBP, HOMA-IRI, and the plasma levels of FPG, TG and LDL-c (P<0.05). The plasma levels of LDL-c of the minor allele-C carriers of the two SNP were both significantly higher than the noncarriers in the control group (P<0.05). Furthermore, insulin resistance index (HOMA-IRI) of the rare homozygotes C/C of rs11868035 was significantly lower than that of T/T in the T2DM group (P<0.05). **Conclusions:** These findings indicate that The SREBP-1c SNPs rs2297508 and rs11868035 are associated with a significantly increased risk of T2DM and dyslipidemia in the Chinese population. Moreover, The SNP (rs11868035) is closely related to insulin resistance (IR) in diabetic patients.

P16-1:

SITE-SPECIFIC GENOMIC INTEGRATION IN HUMAN CELL LINES MEDIATED BY NEW LEVENTIVIRAL VECTOR SYSTEM

Huan-zhang Zhu, Qing-Ting Xin, Shao-hui Liu, Long Yu

State Key Lab of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai, P. R. China

Lentivirals vectors based on HIV-1 are capable of stably inserting transgenes into the host cell genome, allowing maintenance of a therapeutic gene during cell division, Therefore , it has become an important tool for gene transfer in vitro and in vivo. Unfortunately, the insertion events produced by the vector can lead to a risk of insertional mutagenesis. To develop a new lentiviral vector that integrate therapeutic genes at “safe sites” within the human genome, and thereby avoid oncogenesis, Firest, we have developed a nonintegartive lentiviral vector by mutation of HIV-1 integrase (D64 Nand D116N), These double mutant vectors system efficiently transduced dividing cell lines in vitro., while cells transduced with HIV integrase mutant vector resulted in a steady decline of expression, from 85 to 0% of cells by day 10. Second, a new site-specific integrative leventiviral vector was obtained by combining nonintegartive lentiviral vector system with site specific integration phi C31 system. The result show that the expression of enhanced green fluorescent protein was stable during repeated passaging in transduced cells, exogenous gene were integrated into pesuo attP site, which existed in smaller genomics region, by analysis of integration sites. Our results suggest that a new site-specific integrative leventiviral vector were developed, which will be efficient, safety and a valuable tool for based stem cell gene therapy and chromosome engineering.

P16-2:

FEATURE SELECTION FOR MICROARRAY ANALYSIS USING MAXIMAL-OVERLAP ESTIMATOR OF THE WAVELET VARIANCE

Isidro C. Medina Jr., Alex C. Gonzaga, Marilou G. Nicolas

Department of Physical Sciences, College of Arts and Sciences, University of the Philippines, Manila

DNA microarray has been recently developed to study the transcriptome of cells. It has been used in many studies for tumor classification and of identification of marker genes associated with different diseases. However, microarray data often suffers the 'curse of dimensionality' since a very large number of genes are measured against a small number of samples. A general approach to overcome this challenge is to perform feature selection technique prior to classification. Feature selection is an important step in most pattern recognition problems including disease classification using microarray data. In this study, a feature selection approach based on the maximal-overlap estimator of the wavelet variance was devised for microarray analysis. Studies have showed that estimators of the wavelet variance are statistically efficient and effective in analyzing a very wide range of signals and phenomena.

P16-3:

HIGH-THROUGHPUT CELL-BASED SCREENING REVEALS A ROLE FOR ZNF131 IN REGULATING ACTIVITY OF Erα

Xiao Han [1,4], Jinhai Guo [1], Weiwei Deng [1], Dalong Ma [1,2,3], Peige Du [4], Taiping Shi[1,2,3]

[1]Chinese National Human Genome Center, #3-707 North YongChang Road BDA, Beijing 100176, P. R. China; [2]Laboratory of Medical Immunology, School of Basic Medical Science, Peking University Health Science Center, 38# Xueyuan Road, Beijing, 100083, P. R. China; [3]Peking University Center for Human Disease Genomics, 38# Xueyuan Road, Beijing, 100083, P. R. China; [4]Beihua University, 3999 Huashan Road, Jilin City, Jilin Province132013, P. R. China

Background: Estrogen receptor α (ERα) is a transcription factor whose transcriptional activity is affected by multiple regulatory cofactors. In an effort to identify human genes involved in the regulation of ERα, we constructed a high-throughput, cell-based functional screening platform by linking a response element (ERE) with a reporter gene, that enable quantitatively analyzing of the cellular activity of ERα in cotransfected cells in the presence or absence of its cognate ligand E_2. **Results:** From a library of 570 human cDNA clones, we identified zinc finger protein 131 (ZNF131) as repressor of ERα mediated transactivation. ZNF131 is a typical member of BTB/POZ family of transcription factors with ubiquitous expression and is highly conserved during evolution. The luciferase reporter gene assay revealed that ZNF131 inhibits ligand-dependent transactivation by ERα in a dose-dependent manner. Electrophoretic mobility shift assay clearly demonstrated that the interaction between ZNF131 and ERα will interrupt/prevent ERα binding to the estrogen response element (ERE). Moreover, ZNF131 could suppress the expression of pS2, an ERα target gene. **Conclusion:** The functional screening platform we constructed therefore can be applied for high-throughput genomic screening candidate ERα-related genes, which would provide new insights into underlying molecular mechanisms of ERα regulation in mammalian cells.

P16-4:

LIMITATIONS OF GENOME-WIDE ASSOCIATION STUDIES IN CLASSICAL INBRED MICE

Wan-Lin Su[1], Joshua Millstein[2], Solveig K. Sieberts[2], Eugene Chudin[2], Cliona Molony[2], Eric E. Schadt[2]

[1]Molecular and Cellular Biology Program, University of Washington, Seattle, WA 98195, USA; [2]Rosetta Inpharmatics LLC, a wholly owned subsidiary of Merck & Co., Inc., 401 Terry Ave N, Seattle, WA 98109, USA

Recent reports concerning genome-wide association mapping in humans has shown significant success in identifying DNA regions responsible for controlling variation of complex traits linked to diseases. These high-impact publications have renewed interests in using genome-wide association mapping in model organisms such as inbred mice. Originally proposed by Grupe *et al*. in 2001, the main advantage of *in silico* mapping in inbred mice is the increase in resolution (approximately 40-fold) over traditional QTL mapping where large tracts of the genome are identified. In addition, earlier arguments against *in silico* mapping citing limited marker density and lack of genotype information are now less of an issue with the public release of multiple high-density SNP datasets. Nevertheless, the emerging complexity of the genetic population structure of inbred mice suggests that there may be other barriers to performing genotype-phenotype associations at the genome-wide level. Hence, we sought to empirically assess the effects of the genetic population structure present in inbred mice on *in silico* mapping and determine the viability of *in silico* mapping in classical inbred mice strains. Using cis-acting expression quantitative trait (cis-eQTLs) as the gold standard for QTL detection, we first demonstrate the extent of spurious associations present in such studies and secondly, that these signals are indistinguishable from true signals. We then provide empirical evidence that an integrative analysis combining traditional QTL mapping techniques with association mapping results in significantly higher statistical power. Finally, a short discussion on the implications of our findings for *in silico* mapping in inbred mice is presented.

P16-5:

PREDICTIONG DNA-BINDING SITES OF PROTEINS FROM AMINO ACID SEQUENCE USING RANDOM FOREST MODEL WITH HYBRID FEATURES

Jiansheng Wu[1], Hongde Liu[1], Xueye Duan, Yan Ding and Xiao Sun[1]

[1]State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, P. R. China

In this work, we aim at developing a computational approach for predicting DNA-binding sites in proteins from amino acid sequences. In order to avoid over-fitting with this method, all the available data sets of DNA-binding proteins from the Protein Data Bank (PDB) are trained for constructing the models. Random forest (RF) algorithm is used here because it is fast and has robust performance on different parameter values. A novel hybrid feature is presented which incorporates evolutionary information of the amino acid sequence, secondary structure (SS) information and orthogonal binary vectors (OBVs) information which reflect the characteristics of 20 kinds of amino acids on two physical-chemical properties (dipoles and volumes of the side chains). The numbers of binding and non-binding residues in proteins are highly unbalanced, so a novel scheme is proposed here to deal with the problem of imbalanced datasets by downsizing the majority class. A nested cross-validation procedure is adopted in this paper to assure that the parameter estimation and model generation of RFs are completely independent of the test data. The prediction reliability index (RI) is calculated to evaluate the quality of prediction and is likely to help biologists to pick residue candidates in experimental studies of site-directed mutagenesis for functional DNA-binding proteins. The results show that the RF models achieves 91.41% overall accuracy (ACC) with Matthew's correlation coefficient of 0.70 and the area under receiver operating characteristic curve (AUC) of 0.913, respectively. To our knowledge, up to now, the RF method using the hybrid feature is the computationally optimal approach for predicting DNA-binding sites in proteins from amino acid sequences without using three-dimensional structural information. The SVM method was selected as an alternative algorithm to compare with the RF classifier in this study, and the ROC analysis shows the hybrid feature contributes most to the excellent prediction performance. When comparing with previous methods, the results indicate that our RF model achieves the best performance. We have examined the performance of 374 DNA binding and 350 non-binding proteins, and the results suggest that the CI values of DNA binding and

non-binding proteins would be helpful for predicting whether a given protein is a binder or non-binder. We have demonstrated that the prediction results are useful for understanding protein-DNA interactions by detailed presentation of the prediction of two representative protein-DNA complexes.

P17-1:

FNS-INDUCIBLE GTPase PROTEINS LACKING IFN SYSTEM IN AMPHIOXUS

Guang LI, Juyong ZHANG, Hua WANG, Yi SUN, Lu CHEN, Yiquan WANG

School of Life Sciences, Xiamen University, Xiamen 361005, China

IFNs-inducible GTPases, including four families of proteins: Mx, GBP, VLIG and IRG, have been demonstrated to be under conserved regulation by interferon in humans and mice, and play a critical role in preventing microbial infections. However the knowledge about them in other vertebrate species remains relatively poor and their origin is not well addressed yet, although the issues may shed new light on the evolution of IFN system. In the present study, we analyze four IFNs-inducible GTPase families from above ten representative animals and obtain several unexpected results. First, we found that Mx, GBP and IRG protein families radiated in the last common ancestors of vertebrates and cephalochordates, which is prior to the emergence of IFN system in vertebrates. Secondy, these four multigene families had experienced a high rate of gene gain and loss during evolution. Thirdly, the regulation of *Mxs*, *IRGs* and *GBPs* by interferon is highly conserved in all lineages of vertebrates, but for VLIG proteins, this property appeared to be existed only in tetropads and not in fishes. Finally, amphioxus IFNs-inducible GTPase genes are all highly expressed in immune-related organs such as gill, liver and intestine, and were up-regulated after challenge with poly-I:C and pathogens, although it was know that amphioxus does not possess the IFN-mediated signaling pathway. Our results indicate that IFNs-inducible GTPase genes from both vertebrates and amphioxus (except for fish *VLIGs*) are similarly highly expressed in immune tissues, can be up-regulated by injection of pathogens, and play critical roles in defending against microbial invasion. But their regulations are mediated by different molecular system, with vertebrate genes by IFN system and amphioxus genes by an unknown novel system.

P18-1:

IDENTIFICATION AND EXPRESSIONAL CHARACTERISTICS OF TSC29 IN MOUSE AND HUMAN TESTIS

Yongcui Zhou, Aifa Tang, Qiuxia Yan, Bin Yan, Zhendong Yu, Yaoting Gui, Zhiming Cai

Laboratory of Male Reproductive Medicine, Peking University Shenzhen Hospital, Shenzhen 518036, The People's Republic of China.

Background: With the Affymetrix microarray analysis, we identify a testis specific gene, named TSC29. Mouse TSC29 gene is localized in chromosome 6C3. The full cDNA length of mouse TSC29 is 744 bp, with a 615 bp open reading frame encoding a 205 amino acids protein with a predicted molecular weight of 22.99 kDa. Human TSC29 gene is localized in chromosome 1C23.3. The full cDNA length of human TSC29 is 907 bp, with a 786 bp open reading frame encoding a 262 amino acids protein with a predicted molecular weight of 29.16 kDa. Multiple amino acid sequence alignment shows that mouse TSC29 protein was highly homologous with the human and rats. **Objective:** To detect the expressional characteristic of TSC29 in mouse and human testis. Methods: RT-PCR and Western blotting were used to detect the expression of TSC29 in different organs in the mouse and human, as well as in the mouse testis on different days. Immunohistochemistry was used to localize the expression of TSC29 in the mouse and human testis. **Results:** RT-PCR showed TSC29 mRNA was only expressed in the mouse testis after 20 days of birth. RT-PCR and Western blotting showed TSC29 was only expressed in human testis. Compared with normal human testis, the expression of TSC29 was significantly decreased in the patients with spermatogenic arrest. The results from immunohistochemistry demonstrated TSC29 was only located in spermatogenic cells with higher expression in spermatids. **Conclusion:** TSC29 was specifically expressed in the mouse and human testis, its expression was significantly decreased in the patients with spermatogenic arrest. The study in the next will be exploring the function of the gene in spermatogenesis by methods of RNA interference and gene knockout.

P18-2:

MEASUREMENT AND MEANING OF INSULIN-LIKE GROWTH FACTOR-I IN SERUM AND FOLLICULAR FLUID DURING CONTROLLED OVARIAN HYPERSTIMULATION PERIOD

Weng Lanying, Xiao Xiao

Department of Obstetrics and Gynecology ,The First Affiliated Hospital of Nanchang University Jianxi 330006 China

Objective: To observe the relationship among the levels of insulin-like growth factor-I (IGF-I) in serum before and after stimulated and follicular fluid during controlled ovarian hyperstimulation period in vitro fertilization and embryo transfer (IVF-ET); the relationship among IGF-I and gonadal hormone and gonadotrophin in follicular fluid, and assessing the impacts of IGF-I on parameters of IVF-ET. **Methods:** 42 cycles of IVF-ET patients were used the same superovulation regimen. IGF-I concentration in serum and follicular fluid samples were measured using emzyme-linked immunosorbent assay, gonadal hormone [estradiol (E_2), progesterone (P)], gonadotrophin [luteinizing hormone (LH), follicle-stimulating hormone (FSH)] in follicular fluid samples were measured using chemiluminescence analysis. The follicles were divided into three groups: low reactive group, moderate reactive group, and high reactive group according to the number of follicle that diameter≥14mm on OPU day. Register the numbers of clinical pregnancy and the correlated parameters (number of obtained eggs, number of oosperm, fertility rate, number of cleavage, cleavage rate). **Results:** 1. There were no significant changes among the levels of IGF-I in serum before and after stimulated and follicular fluid in low reactive group ($p>0.05$). Also as in the moderate reactive group and in high reactive group, there were no significant changes among the levels of IGF-I in serum before stimulated in three groups ($p>0.05$), Also as in serum after stimulated and in follicular fluid. 2. In pregnancy group and non-pregnancy group, the difference of IGF-I in follicular fluid were non-significant ($p>0.05$). The difference of E_2 were significant ($p<0.05$). The difference of P were non-significant ($p>0.05$). The difference of LH were significant ($p<0.05$). The difference of FSH content was non-significant ($p>0.05$). 3. Follicular fluid IGF-I level was positively correlated with E_2 ($r =0.38$, $p <0.05$), it was nondistinctive correlation with P($r =0.32$，$p >0.05$), it was positively correlated with LH

(r=0.45,p＜0.05), while it was negatively correlated with FSH (r =-0.31, p <0.05). 4.In follicular fluid, IGF-I was nondistinctive correlation with numbers of obtained egg (r=0.25, p>0.05), numbers of oosperm (r=0.36,p>0.05) and number of cleavage (r=0.31, p>0.05). It was positively correlated with rate of oosperm (r=0.31, p>0.05) and rate of cleavage (r=0.42, p<0.05). **Conclusion:** IGF-I exists in follicular fluid, which could come from peripheral blood circulation and may be worked in adjusting the process of follicle growth. IGF-I contributed the synthesis and secretion of E_2 and LH, and at the same time it could coordinate the gonadotrophin secretion. It is not significantly effect on pregnancy, while has certain relationship with the development of fertilized eggs.

P18-3:

EXPRESSION AND FUNCTION OF STAT1 IN DECIDUAS OF EARLY NORMAL PREGNANCY AND SPONTANEOUS ABORTIONS

He Junlin, Dong Yanling, Liu Xueqing, Ding Yubin, Wang Yingxiong

Department of Genetics, Chongqing Medical University, China

Objective: The analysis of genetic expression profile in spontaneous abortion deciduas with applied gene array found that signal transducers and activators of transcription 1 gene (STAT1) was one of the significant up-regulation expression genes. STAT1 protein that is regarded as a key link of JAK/STAT1 signal transduction pathway plays an important role in adjusting transcription and expression processes of relevant gene. A number of studies have identified that lots of cytokines, such as IFN, IL-2, IL-4, IL-6, CNTF and growth factors, such as EGF, PDGF, CSF utilize this signal transduction pathway to induce cell proliferation, differentiation or Apoptosis. But there is no research to explore effect of STAT1 in early pregnant deciduas. On the basis of our previous research, this study aims to identify STAT1 expression in deciduas from normal pregnancy and spontaneous abortion at gene level and protein level respectively, in order to explore the role of STAT1 in implantation and spontaneous abortion. **Methods: 1. Group of decidua from normal pregnancy:** (1) Collecting decidua of artificial abortion during the 6th week to 11th week of early normal pregnancy respectively. (2) Freezing decidua at -80□ and extracting total RNA of each decidua, then detecting STAT1 mRNA expression of the decidua samples by RT-PCR. (3) The decidua was fixed by 4% of Polyoxymethylene, imbedded by paraffin and sliced, and then observing STAT1 expression directly by immunohistochemistry. (4) Freezeing decidua at -80□ and extracting total protein of each decidua, and then detecting STAT1 protein expression of the decidua samples by western blot. (5) The decidua was fixed by 4% of Polyoxymethylene, imbedded by paraffin and sliced, and then observing STAT1 mRNA expression directly by in-situ hybridization. (6) The decidua was fixed by 4% of Polyoxymethylene, imbedded by paraffin and sliced, and then observing STAT1 expression directly by immunofluorescence. **2. Group of decidua from spontaneous abortion:** (1) Collecting decidua from spontaneous abortion respectively, including missed abortion、inevitable abortion and threatened abortion (during the 6th week to 11th week of early pregnancy). (2) The decidua were analyzed by RT-PCR, immunohistochemistry, western blot, in-situ hybridization and immunofluorescence.

Results: 1. Group of decidua from normal pregnancy: (1) The results indicated that STAT1 mRNA expression in deciduas showed a gradual descent trend with days past by RT-PCR. (2) The results from immunohistochemistry had identified STAT1 expression in decidua cell's endochylema. STAT1 expression was the strongest in the 6^{th} week of pregnancy, but the most weak in the 11^{th} week of pregnancy. (3) The findings from western blot were similar with what have been found by RT-PCR, that STAT1 protein expression showed gradual descent trend with days past. (4) The findings of in-situ hybridization showed that hyacinthine grain distributed in endochylema. The dye was the deepest and a positive area was the most widespread in the 6^{th} week of pregnancy, but the most poor in the 11^{th} week of pregnancy. (5) The results from immunofluorescence indicated that fluorescence signal in decidua was the most strong in the 6^{th} week of pregnancy and then showed gradual descent trend with days past. The fluorescence signal in decidua was the most poor in the 11^{th} week of pregnancy. **2. Group of decidua from spontaneous abortion:** (1) The findings from RT-PCR showed that there is no significant difference on STAT1 mRNA expression in decidua from three kind of samples of spontaneous abortion, including missed abortion、inevitable abortion and threatened abortion, but their photo-density were stronger than that of decidua from normal pregnancy. (2) The results from immunohistochemistry showed that brown grain widely distributed in endochylema and cell nucleus of three kind of decidua from spontaneous abortion, and their color were deeper than that of deciduas from normal pregnancy. (3) The findings from western blot showed that there is no difference on STAT1 protein expression in three kind of decidua from spontaneous abortion, but their expressions were stronger than that in decidua from normal pregnancy. (4) The results from in-situ hybridization showed that hyacinthine grain widely distributed in endochylema and cell nucleus and their photo-density were significant stronger than that in deciduas from normal pregnancy. But there was no significant difference among three kind of decidua from spontaneous abortion. (5) The findings from immunofluorescence showed that fluorescence signal in deciduas from spontaneous abortion were significant stronger than that in deciduas from normal pregnancy. But there was no significant difference among three kinds of deciduas from spontaneous abortion. **Conclusion:** Semi-quantization RT-PCR、immunohistochemistry、in-situ hybridization、western blot and immunofluorescence were applied to detect STAT1 expression in deciduas from spontaneous abortion and normal pregnancy. It showed that STAT1 expression showed gradual descent trend with days past in decidua from normal pregnancy during the 6^{th} week to the 11^{th} week, but STAT1 expression was significant stronger in decidua from spontaneous abortion than that in decidua from normal pregnancy. These results suggested that STAT1 might play an important role in the implantation.

Abnormal increasing of STAT1 expression may affect implantation through some mechanisms, and then further induce abortion.

P18-4:

THE EXPRESSION RULE AND SIGNIFICANCE OF CALRETICULIN OF EARLY PREGNANCY

He junlin, Dong Yanling, Liu Xueqing, Ding Yubin, Wang Yingxiong

Department of Genetics, Chongqing Medical University, China

Embryo implantation is complicated process of reproductive and physiology which is associated with differentiation of blastocyst development, synchronization of endometrium, gestation reaction and blastocyst adhesion/invasion into uterine endometrium. Blastocyst adhesion/invasion into uterine endometrium are two important event of embryo successful implantation. During the initial steps of implantation, the mouse uterine epithelium of the implantation chamber undergoes apoptosis in response to the interacting blastocyst. With progressing implantation, regression of the decidual cells allows a restricted and coordinated invasion of trophoblast cells into the maternal compartment. The progressing studies on the molecular mechanism of embryo implantation revealed that a number of cytokines, adhesion molecule formed a network to regulating the complex process. Calreticulin is a multifunctional $Ca2^{+}$-binding protein of the ER shown to be important in $Ca2^{+}$ homeostasis, modulation of gene expression, apoptosis and cell adhesion. Overexpression of calreticulin increases both cell to substratum and cell-to-cell adhesiveness. Furthermore, CRT can also modulate integrin-dependent $Ca2^{+}$ signaling and steroid-sensitive gene outside the endoplasmic reticulum. However, integrin signaling is critical for blastocyst adhesion to endometrial ECM during implantation and may modulate adhesive interactions with ECM components as trophoblast cells pass through the basal lamina, invade the decidua and remodel the uterine vascular system. According to the research finding above, it is postulated that calreticulin might play an important roles in blastocyst implantation. However, there is no direct evidence to support this conjecture in the mammalian species. This study is to investigating the role of calreticulin in the process of embryo implantation using RT-PCR, confocal laser scanning Microscopy and hybridization in situ techniques. **Objective:** To investigate the expression rule of CRT in the mice endometrium and analyze its role in blastocyst implantation. **Methods:** 1. Endometrium from day1 to day7 in early pregnancy mice were used to following studies. Both non-pregnant (d0) and pregnant mice (d1, d2, d3, d4, d5, d6, d7) were randomly divided into 8 groups and there were 40 mice for each group. RT-PCR, indirect immunofluorescence histochemistry, western blot and hybridization in situ techniques have been applied to detect CRT mRNA and protein expression in endometria from non-pregnant mice and

pregnant mice on day1 to day7. 2. The number of blastocyst implantation was registered by injecting CRT antisense oligonucleotide in horn of uterus at 20:00 on the pregnant d3. **Result:** 1. Expression of CRT on endometrium of mouse during early pregnancy. 1.1 The result showed that The expression of CRT mRNA in pregnant endometria is higher than that of non-pregnant ($P < 0.05$), which showed a gradual raise trend with pregnant days passed from day1 to day5 and reached the maximum level on pregnant day5. 1.2 The result of hybridization in situ is identical with that of RT-PCR and positive expression of CRT was located in the cytoplasm of epithelia and stromal cells. 1.3 By indirect immunofluorescence histochemistry, the positive expression of CRT protein has been observed in endometria of pregnant and non-pregnant mice and mainly located in the cytoplasm of epithelia and stromal cells. The CRT expression of endometria in pregnant is higher than that of non-pregnant and reached the maximum level on pregnant day5. On pregnant day6 to day7, the expression level of CRT protein is down-regulated, but there are no statistically significant difference compared with d4 and d5. 1.4 The result of western blot is concordance with that of the immunofluorence histochemistry. 2. The numbers of blastocyst in horn of uterus which was injected CRT antisense oligonucleotide is fewer than that of injecting saline. **Conclusion:** 1. According to the expression rule and distribution of CRT mRNA and protein on mouse endometrium during early pregnancy, we suggest that CRT might be involved in the initial adhesion of blastocyst implantation. It influences the action between trophoblast and endometrium to make the best reception of endometrium for the blastocyst implantation by affecting adhesion of integrin. 2. CRT antisense oligonucleotide was used to hold back its transcribe and to furthermore block up its physiological function. This result of this study shows that number of embryo implantation where injected antisense oligonucleotide is fewer than that of injecting saline. It suggests that CRT might play an important role.

P18-5:

A CASE OF AZFB PARTIAL DELETION WITH MATURE SPERM IN SEMEN

LI Hong-Gang, XIONG Cheng-Liang

Family Planning Research Institute, Tongji Medical College, Huazhong University of Science and Technology, HangKong Rd. 13, Wuhan 430030, China

Yq microdeletions are routinely screened in many laboratories worldwide. The guidelines for AZF microdeletions were published. AZFa, AZFb, AZFb+c or AZFa+b+c deletion was reported to be associated with the testis phenotypes of Sertoli-Cell-Only Syndrome or maturation arrest. But the occurrence of these deletion subtypes are low, further confirmation is needed. Our preliminary study utilized 15 sequence tagged sites (STS), in which 6 STS loci recommended by European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) were included, to detect deletion of AZFa, AZFb and AZFc in 80 Chinese patients with severe oligozoospermia. Unexpectedly, a patient shows the deletions of both sY127 and sY134, which were recommended by EAA/EMQN for the complete deletion of AZFb and the corresponding testis phenotype, should be Sertoli-Cell-Only Syndrome or maturation arrest. Our further breakpoints mapping suggests the patient possess a new subtype of AZFb deletion caused by recombination between b5 and b2.

P18-6:

A STUDY ON THE SCREENING OF THE SEX-SPECIFIC SEQUENCE OF CANNABIS SATIVA L.

Pei Li[1], Zhang Guiqin[2], Tu Zheng[1], Peng Jianxiong[1], ZhangYing[1], Xuxiaoyu[1], GuoJia[2]

[1]Instiitute of Forensic Science, Ministry of Public Security, Beijing, 100038,China; [2]Chinese People's Public Security University, Beijing 100038, China

Cannabis sativa L. is a dioecious plant. There are no or few THC and CBD in the leaves and stem in the male hemp, whereas, the female hemp has much THC and CBD components. Therefore, the female plant is considered to have the medical value and the potential for drug abuse. It is difficult to identify the male or female plants in the infant phase of the plant. So the best occasion of rooting them out will be missed after their flowering. Thus, the early identifying the sex of the hemp plant play an important role in terms of beating at drug crimes. we searched the GENEBANK and selected the code sequences AJ864397, AJ864398, AJ864399, AJ864400 and designed primer for them respectively. We test the specificity of them in identifying the sex of the hemp plants. The primers DM016 and DM029 were finally selected for identifying the sex of the hemp plants according to the comprehensive consideration to the specificity, the anneal temperature of PCR and amplified fragment length. We did much experiment to test the 276 hemp samples from Yunnan, Xinjiang, Gansu, Shanxi province. The results showed that the accuracy was up to 96%.

P18-7:

CHROMOSOME ANOMALIES AND Y-MICRODELETIONS IN SUBFERTILE MEN

Paulina Ng[1], Mary Tang[2], Elizabeth Lau[2], Lucy Ng[2], Ernest Ng[1], PC Tam[3], William Yeung[1], PC Ho[1]

[1]Department of Obstetrics & Gynaecology, The University of Hong Kong, Hong Kong; [2]Prenatal Diagnostic & Counselling Department, Tsan Yuk Hospital, Hong Kong; [3]Department of Surgery, The University of Hong Kong, Hong Kong

From 2000 to 2007, three hundred and one Chinese subfertile men with azoospermia or severe oligospermia (<5 million/ml) underwent somatic chromosome analysis and Y-microdeletion studies. Cytogenetic analysis was based on Giemsa banded metaphases from peripheral blood lymphocytes. Molecular analysis of the AZF region of the Y chromosome was performed on DNA extract from peripheral blood by polymerase chain reaction (PCR) using six Y-chromosome specific-sequence tagged site (STS) markers: sY84, sY86 (AZFa region); sY127, sY132 (AZFb region); sY254, sY255 (AZFc region). The type and frequency of chromosome anomalies and Y-microdeletions were analysed. Ten cases involved sex chromosome aneuploidies (5 cases of 47,XXY, 1 case of 47,XYY, 4 cases of mosaic 47,XXY). Eight cases revealed structural alterations of the Y chromosome with five cases having AZFb and c deletion. Fifteen cases had isolated AZFc deletion. Eleven cases showed autosomal chromosome anomalies. The incidence of chromosome anomalies and Y-microdeletions detected were 9.6% (29/301) and 6.6% (20/301) respectively. Subfertile men with chromosome anomalies and Y-microdeletions were counselled by clinician specialists on assisted reproduction treatment.

P18-8:

MUTATION SCREENING OF PICK1 GENE IN FOUR CHINESE PATIENTS WITH GLOBOZOOSPERMIA

Shi QW, Liu G, Lu GX

Institute of Human Reproduction and Stem Cell Engineering, Central South University

Introduction: The acrosome plays an important role at the site of sperm-zonapellucida binding during the fertilization process. Globozoospermia whose most prominent feature is the malformation of the acrosome is a human infertility syndrome caused by spermatogenesis defects (OMIM102530). Several kinds of knockout mouse model demonstrated a phenotype similar to globozoospermia in human. But no mutations with a clear link to globozoospermia were found in human homologs. In the protein interacting with C kinase 1（PICK1） knockout mouse model, a phenotype similar to globozoospermia in human was found. We screened all the exons of PICK1 in 4 Chinese patients with globozoospermia. **Materials/methods:** The four patients have 100% round-headed spermatozoa. Genomic DNA was extracted from peripheral blood leukocytes of the patients. Polymerase chain reaction (PCR) and DNA sequencing were applied to detect the mutations in all exons of the PICK1 gene. PCR-restriction fragment length polymorphism (PCR-RFLP) was applied to affirm the mutation detected by DNA sequencing. **Results:** We have identified a homozygous missense mutation G98A in exon 13 of the PICK1 gene causing a mutation G393R in the protein from a Chinese family with globozoospermia type I, in which the affected member showed the complete lack of acrosome. The PCR-restriction enzyme digestion showed that the PvuII enzyme digestion location disappeared in the patient, which verified the results of sequencing analysis. **Conclusion:** Up to now, five mouse genes have been proved to be associated with globozoospermia by the knockout out mouse models. But the mutation we found in PICK1 is the first reported mutation with a clear link to globozoospermia in human homologs while no mutations were found in the other four genes in recent years.

P18-9:

EXPOSURE TO 17A-ETHYNYLESTRADIOL IMPAIRS REPRODUCTIVE FUNCTIONS OF BOTH MALE AND FEMALE ZEBRAFISH (DANIO RERIO)

Hui Xu

Department of Molecular Genetics, Fudan University Shanghai Medical School, 138 Yixueyuan Road, Shanghai, P.R.China, 200032

In this study, the impact of 17α-ethynylestradiol (EE2) on reproduction in zebrafish (Danio rerio) was evaluated using vitellogenin (Vtg) induction, mortality rate, growth, sex ratio, gonad histology, fecundity, and sperm parameters as endpoints. Two days post-hatch (2 dph) zebrafish were exposed to solvent control or EE2 at 0.4, 2, and 10 ng/l for 3 months. At 21 dph, Vtg mRNA expression was detected only in fish exposed to 10 ng/l EE2. At 90 dph, increased mortality rate and sex ratio (female:male) were observed in fish exposed to 2 and 10 ng/l EE2. A dose-dependent increase in gonads with underdeveloped gametes was observed in fish exposed to EE2. At 180 dph, malformation of the sperm duct and reduced number of spermatozoa were found in fish exposed to 2 ng/l and 10 ng/l EE2. Reduced fecundity and 12 hpf egg viability were found in EE2-exposed males and females. The number of fish with no expressible milt was elevated dose dependently in EE2-exposed males, although no difference in sperm density was found. After a 3-month recovery period, growth and sex ratio were partially recovered. Our findings suggest that EE2 can adversely affect the fecundity, sex differentiation, gametes development, and other reproductive functions of both male and female zebrafish, and some of the toxic effects persist.