

**Abstracts of the 41st Annual Meeting of
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Plenary Lectures**PL-2****DNA REPAIR DEFECTS IN MUTAGENESIS AND CARCINOGENESIS.****Mutsuo SEKIGUCHI** (Dept. Biol., Fukuoka Dental College, Fukuoka)

There is a special class of mutations that increases the frequency of spontaneous mutation. These are collectively termed mutators and are useful tools for elucidating cellular mechanisms related to the high fidelity of DNA replication. Once a mutant with a mutator phenotype is isolated, it can be used to clone the gene responsible and the gene product can be identified. This in turn paves the way for determination of the biochemical nature of the gene product and further elucidation of its role in the entire process of replication. These proteins may be responsible for genomic stability and defects in the genes would cause an increased frequency of occurrence of cancers.

PL-3**HUMAN GENETICS--- PAST, PRESENT, AND FUTURE****Toshiyuki YANASE** (Prof. Emeritus, Univ. Kyushu, Fukuoka)

This is a special lecture commemorating the 40th anniversary of the founding of the Japan Society of Human Genetics. The following items are covered: Human genetics in the earlier days and four pioneering studies in Japan/ Establishment of the Japan Society of Human Genetics (1956)/ Monumentary studies at the dawn of a new age- those in human population genetics, cytogenetics, and biochemical genetics/ Genetic epidemiology and statistical genetics particularly involving analysis of family data and genetic linkage/ Inborn errors of metabolism discovered in Japan/ Studies on biological effects of inbreeding and genetic load (1960-1970)/ Some indications of new current-discoveries of major histocompatibility antigens (HLA), Philadelphia chromosome, somatic cell fusion, two hit theory in carcinogenesis, etc. (1955-1970)/ Introduction of technologies of gene manipulation into medical biology/ New knowledges of interactions between allelic or non-allelic genes/ Heterogeneity at the DNA level of mutation and molecular pathology of single gene disorders/ Classification of single gene disorders based upon new genetic concepts/ Genomic rearrangement-programmed and unprogrammed rearrangement/ Approaches to the analysis of molecular basis of common polygenic diseases/ A mile stone of gene mapping- assignment of genetic locus responsible for Huntington's disease by RFLP linkage/ Human genome project- present and future directions/ Gene diagnosis and therapy/ Estimation of genetic risks and informed consent in the recombinant DNA era/ Ecogenetic and xenogenetic problems in the recent past, present, and future, with special reference to genomic changes in microbes and man.

PL-4

Molecular pathogenesis of nonketotic hyperglycinemia. Shigeo Kure (Dept. Biochem. Genet., Tohoku Univ. School of Medicine, Sendai)

Nonketotic hyperglycinemia (NKH) is a metabolic disorder with autosomal recessive inheritance, causing severe neurological symptoms. The metabolic lesion of NKH resides in the glycine cleavage system (GCS), a complex enzyme system with four components; P-, T-, H-, and L-protein. The enzymatic analysis revealed that more than 80% of the patients with NKH are deficient of P-protein activity. We cloned the P-protein cDNA and its genomic counterparts. P-protein gene spanned more than 130 kbp and had 25 exons. Several mutations were identified: One missense mutation accounts for 70% of the mutant alleles in Finland, where the incidence of NKH is unusually high. To elucidate the neuropathogenesis of NKH we examined structure and expression of the GCS in rat brain and primary cultured neurons and astrocytes. Those studies supports the hypothesis that the neurological disturbance in NKH is caused by excitoneurotoxicity through the NMDA receptor, which is allosterically activated by high concentration of glycine.

PL-5

MOLECULAR BASIS OF ZELLWEGER SYNDROME, β -KETOTHIOLASE DEFICIENCY AND MUCOPOLYSACCHARIDOSES. Tadao ORII (Chubu Women's College, Seki)

Zellweger syndrome is a fatal autosomal recessive disease, with clinical evidence of severe neurologic abnormalities, dysmorphic features, hepatomegaly, and multiple renal cysts. We isolated three different types of peroxisome-deficient mutant Chinese-hamster-ovary cells—Z24, Z65, and ZP92—as models for human groups E, F, and C, respectively, and clarified that mutation in PAF-1 is the primary defect in the group F patient. We recently cloned human PAF-2 cDNA that restores peroxisomes of group C Zellweger fibroblasts and identified two pathogenic mutations in the PAF-2 gene in two patients with group C Zellweger syndrome. β -Ketothiolase deficiency is a deficiency in mitochondrial acetoacetyl-CoA thiolase (T2). Seventeen mutations were identified in 13 T2-deficient patients. We have identified the first different exonic mutations causing β -glucuronidase deficiency in three unrelated Japanese families with mucopolysaccharidosis VII, and the first two kinds of exonic mutations causing GalNAc6S sulfatase deficiency: one is the two base pair deletion which causes a frame shift (1342delCA) found in a classical Morquio patient and the other is a point mutation (N204K) which destroys one of the two asparagine-linked glycosylation sites, as detected in two mildly affected siblings with mucopolysaccharidosis IVA.

Luncheon Seminars

LS-1

APOPTOSIS OF HEPATOCYTES IN GENETIC DISEASES

Fumio Endo (Department of Pediatrics Kumamoto University School of Medicine, Honjo 1-1-1 860 Japan)

Many genetic disorders involve impairment of metabolic activities in hepatocytes. Some of these cause hepatocytes injury, typically seen in diseases such as galactosemia, fructose intolerance, Wilson disease, α -1 anti-trypsin deficiency. The hepatocytes in the patients with these diseases seem to undergo premature death due to chemical substances formed in the hepatocytes. However precise mechanisms of hepatocyte death under such conditions have not been elucidated. Among these, hereditary tyrosinemia I (HTI) is characterized by progressive liver damage from infancy and by a high risk for hepatocellular carcinomas. HTI is caused by the mutations in the fumarylacetoacetate hydrolase gene *FAH*, encoding the last enzyme in the tyrosine catabolic pathway. To investigate the hepatocytes injury in HTI we developed a mouse model which carried inactivating mutations in both *FAH* gene and *HPD* gene, in which the later mutation rescues the perinatal death due to *FAH* defect. The double mutant *FAH*^{-/-} *HPD*^{-/-} mice appeared normal, at least until age 18 months and with no evidence of liver disease. The hepatocytes of *FAH*^{-/-} undergo rapid apoptosis by retrieval of *HPD* function and acute death follows. Apoptosis of hepatocytes in HTI has implications in treatment and mechanism for liver pathology of genetic liver diseases.

LS-2

MOLECULAR GENETICS OF ESSENTIAL HYPERTENSION.

Ituro INOUE (Human Genetics, University of Utah)

Human hypertension is a very common, heterogeneous disorder. In the recent study, angiotensinogen (*AGT*) gene has been implicated in human essential hypertension (EH) through both genetic linkage and allelic association. In particular, the substitution of a threonine for a methionine at amino acid residue 235, denoted 235T, was found to be associated with EH both in Caucasian and Japanese. Furthermore 235T is associated with elevated plasma *AGT*. In the course of a systematic mutation search in the *AGT* gene, we have identified a variant in the proximal promoter in tight linkage disequilibrium with T235, adenine instead of guanine, six residues upstream from the transcription start site, denoted -6A. Consequently all association described between 235T and EH or plasma *AGT* extends directly to this promoter variant. Since biochemical analysis such as a kinetic study or a pulse-chase experiment shows a negative impact on 235T, our functional test of *AGT* was targeted to a transcriptional activity due to the variation in the promoter. Two segments of *AGT* promoter were fused to luciferase gene, and activity of this enzyme after transfection in HepG2 served as a reporter of promoter activity. Detailed statistical analysis of the data revealed a highly significant difference in the promoter activity, although difference was small. Also presence of a specific DNA binding protein in the region that behaves as a negative regulator was confirmed. These results reveal that the nucleotide substitution at position -6 affects the transcriptional activity of *AGT*, which could lead to the development of EH.

LS-3

ETHICAL, LEGAL AND SOCIAL ISSUES IN HUMAN GENETICS. Hiraku TAKEBE (Dept. Radiat. Genet., Fac. Med., Kyoto Univ., Kyoto)

Recent rapid progress in human genetics and human gene studies appears to be changing the concept of medicine. Gene diagnosis can predict certain diseases far before the development of the symptoms. Gene therapy may be able to treat some diseases which have been regarded intractable. From the biological view, however, the diversity of genes, or the genome diversity, is the basic characteristics of all living organisms, and human beings are no exception. Every person should have at least several recessive genes which might seriously affect one's health in the homozygous state. Mutations can take place at certain probability in all genes. Human beings must live with the existing human genome, and to abolish genetic diseases should not be the goal of human genetics. To discuss ethical, legal and social issues (ELSI) in human genetics, "all human beings are born free and equal in dignity and rights", as stated in the United Nations Declaration of Human Rights (1948) must be the starting point. The Human Genome Organization (HUGO) established ELSI Committee in 1992 and Statement on the Principled Conduct of Genetic Research, emphasizing 10 "C"s, communication, consent, confidentiality, etc. was published in 1996.

LS-4

INTRA-BODY EVOLUTION OF PATHOGENIC VIRUSES WITH SPECIAL REFERENCES TO HIV AND HCV

Takashi GOJOBORI (National Institute of Genetics, Mishima)

It is now known that the rate of nucleotide substitution for the genes of HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus), is a million times higher than that for human genes. This characteristic feature of pathogenic viruses gives us a unique opportunity to observe the generating mechanism of genetic variability, even within a single host. Indeed, it is rather common practice to obtain a viral isolate from a single patient or carrier at several time points after infection. The isolated viral clones have been sequenced and the nucleotide sequences compared with each other to estimate the numbers of nucleotide substitutions. By using the substitution numbers, we successfully constructed a phylogenetic tree for the clones isolated from a single host. The phylogenetic tree obtained showed that the homogeneous populations of viral clones present at the initial stage, gradually accumulated genetic variability within the human body. We also found that there was a particular period of time when amino acid substitutions were significantly favored. This implies that some sort of positive selection is taking place during this time, in order for viruses to escape from the immune system of the host. These results will be useful in analyzing the evolutionary interaction between the viruses and their hosts.

LS-5

DEVELOPMENTAL AND MORPHOLOGICAL PLANS OF MAMMALIAN VISCEROCRANIUM. Shigeru KURATANI, Isao MATSUO, and Shinichi AIZAWA (Dept. Morphogenesis, IMEG, Kumamoto Univ. School of Medicine, Kumamoto)

The vertebrate skull is composed of the neurocranium and the viscerocranium. The latter is derived from the cephalic neural crest and so is the prechordal part of the neurocranium. A view we favor considers the prechordal cranium as the premandibular viscerocranium, and the whole skull to consist of the neural crest-derived viscerocranium and the mesodermal neurocranium. Of the mammalian viscerocranium, the mandibular arch skeleton undergoes a striking modification through development as seen in middle ear ossicles. The *Hox* code which is known to function in specification of the viscerocranium does not extend rostrally into the mandibular and premandibular segments. By genetic manipulation of rostrally expressed non-*Hox* homeobox genes, the patterning mechanism of the head is demonstrated to be more complicated than isomorphic registration of the *Hox* code to pharyngeal arches. The phenotype of *Otx2* haplo-insufficiency implies the premandibular (prechordal) cranium shares a common specification mechanism with the mandibular arch. Our interpretation of the metameric plan of the viscerocranium offers a new scheme of molecular codes associated with the vertebrate head evolution.

LS-6

A NEW TECHNOLOGY FOR CANCER DIAGNOSIS —TELOMERASE—. Kazuma OHYASHIKI¹, Junko H. OHYASHIKI¹ and Mitsuo OSHIMURA². (¹First Dept. Internal Med., Tokyo Med. Coll., Tokyo, ²Dept. of Mol. & Cell Genet., Sch. of Life Scis., Fac. Med., Tottori Univ., Tottori)

Telomeres are very important structures that protect chromosomal ends from recombination, but the structure itself induces irreversible reduction without the presence of telomerase activity. Researchers are now collecting evidence of telomere dynamics in clinical samples, but we are still far from being able to discuss telomere dynamics and chromosome changes in clinical samples. Although there is a growing body of evidence that telomerase might be a very important key enzyme to obtain immortality, and possibly oncogenesis, other pathways for immortal characteristics are currently being reported. Thus, telomerase is considered to be a new marker of neoplasias. We present the conventional TRAP (telomeric repeat amplification protocol) and a new technique, including fluorescent TRAP that makes it possible to detect telomerase activity semi-quantitatively and *in situ* TRAP assay that allows us to determine the exact telomerase-positive cells. Our current techniques would provide further informations in clinical oncology.

Symposium

S-I-1

FLUORESCENCE *IN SITU* HYBRIDIZATION: APPLICATION ON GENOME MAPPING AND THE CYTOMOLECULAR GENETICS OF CANCER

Johji INAZAWA (Dept. of Hyg. Kyoto Pref. Univ. of Med.)

For establishment of a high-resolution ordering system elongated prophase chromosomes were prepared and applied them to multi-color FISH. This prophase FISH ordering system gives information about orientation as well as order of adjacent DNA loci that are as little as about 100 kb apart. This technique can bridge the gap between the genomic distances analyzed by pulsed-field gel electrophoresis (PFGE) or YAC technologies. FISH to interphase nuclei with chromosome-specific probes is being used frequently to analyze chromosome aberrations in neoplastic tumors. Losses of loci on chromosome 1p were examined in brain tumors using double-target FISH. The 1p loss was found in all of the samples from oligodendrogliomas examined, whereas our series of astrocytic tumors showed a low incidence of deletions of this region. These results strongly suggest that at least one tumor suppressor gene responsible oligodendroglial tumors lies on the short arm of chromosome 1. For the clinical point of view, we investigated FISH as a tool for diagnosis of several types of solid tumors. Of them we examined numerical aberrations of chromosomes 1, 11, and 17 by multicolor FISH of selected probes to cell nuclei derived from fine-needle biopsies of 106 breast tumors. None of the benign tumors and phyllodes tumors showed evidence of aneusomy for each chromosome. However, 71 of the 74 breast cancers (95.9%) for which sufficient material was available showed aneusomic for at least one of the three chromosomes tested. These results suggest that FISH analysis of fine-needle aspiration biopsies can be a practical and useful method for diagnosis of carcinoma in breast tumors.

S-I-2

GENOMIC IMPRINTING AND ITS RELEVANCE TO GENETIC DISEASES. Norio NIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Genomic imprinting is a biological phenomenon determined by an underlying system that may control harmonious development and growth in mammals. It is also relevant to some genetic disorders in man. Since 2 regions, 15q11-q13 and 11p15.5, have well been analyzed among 16 UPD chromosomes, where Prader-Willi syndrome (PWS)/Angelman syndrome (AS) and Beckwith-Wiedemann syndrome (BWS) regions are located, I reviewed recent cytogenetic/molecular genetic findings on these syndromes, especially focusing on imprinting centers. There have been a few PWS/AS patients with imprinting mutations. Similar findings were obtained in a few BWS patients. From the findings in these exceptional cases, an imprinting center (IC) hypothesis was proposed: normally, IC may suppressively control the disease locus or a putative imprinting switch initiation site (SIS), and a mutation in IC may result in a failure of the control, thus leading to the diseases. The IC for PWS/AS may exist at around the *SNRPN*-exon 1 and that for BWS may be the p57^{KIP2} gene itself. As for the IC at 15q11-q13, in the normal female germline, the paternal IC suppresses SIS function, and therefore the paternal imprint would switch to the maternal imprint, whereas in the normal male germline, since IC on the maternally-derived chromosome is inactive, SIS would mark the chromosome with the paternal imprint. This model was successful to explain complex genetic findings on PWS/AS patients. Regarding the IC at 11p15, we recently identified a nonsense mutation in the p57^{KIP2} gene in 4 of 15 familial or sporadic BWS cases. Furthermore, in one family, the phenotypically normal mother had the same mutation as the patient, the findings being consistent with the maternal transmission of mutation in familial BWS cases and with the characteristic of the IC. Further studies on how this gene interacts with *IGF2* are required.

S-I-3

BEHAVIOR OF THE HUMAN *XIST* GENE IN MOUSE CELLS. Ikuva YOSHIDA and Nobuo TAKAGI
(Res. Ctr. Mol. Genet. and Grad. Sch. Envir. Earth Sci., Hokkaido Univ.)

CF150, a mouse-human somatic hybrid cell line, retains one or more inactive human X chromosome(s) as the only human element. This X chromosome was judged to be inactive because it replicates late in S phase, and the *XIST* gene is expressed while several X-linked genes, which are usually subject to inactivation, are not expressed. We, however, could not find in this hybrid cell line any perinuclear-localized sex chromatin body (SCB) at interphase, and chromosome(s) markedly understained with an anti-acetylated histone H4 antibody at metaphase, two of important traits characterizing the inactive X chromosome in mammals. Apparently, the X chromosome remains inactive without SCB formation and hypoacetylation of histone H4, and certain species-specific factors are involved in the manifestation of these characters. In agreement with this view, the X chromosome transferred to HeLa cells was hypoacetylated and formed SCB. We introduced the human inactive X chromosome into mouse embryonal carcinoma cells by microcell fusion to further explore interspecific difference. The human X chromosome was reactivated in monochromosome hybrids, while the *XIST* gene continued to express actively. The methylation status of 5' region of the *XIST* gene varied considerably in different hybrid clones. Thus, it is evident that the X chromosome inactivation is a multi-step phenomenon diverged, to a certain extent, among different mammalian species. It is also envisaged that the inactivation is maintained predominantly by a single condition such as heterochromatinization or replication asynchrony, or collectively by a multitude of attributes so that the breakdown of only a few of them may not entail activation.

S-I-4

CELLULAR SENESENCE AND TELOMERASE. Mitsuo OSHIMURA
(Dept. Mol. Cell Genet., Sch. Life Scis., Fac. Med., Tottori Univ., Tottori)

Hybrids between immortal cells and normal cells senesce, indicating that immortal cells have lost, mutated or inactivated genes that are required for the program of senescence in normal cells. Genes involved in the senescent program have been mapped to 10 different loci by introduction of human chromosomes via microcell fusion. Multiple pathways of cellular senescence have also been demonstrated by chromosome transfer, indicating that the functions of the senescence genes mapped to different chromosomes are probably variable. One possibility is that one or more of the senescence genes defined by chromosome transfer may suppress telomerase activity in tumor cells, resulting in telomere shortening and cellular senescence. To test this hypothesis, we examined telomerase activity and the length of terminal restriction fragments (TRFs) in microcell hybrids by introducing single human chromosomes into various tumor cell lines. Reintroduction of a normal chromosome 3 into some renal cell and lung carcinoma cell lines with loss of the short arm of chromosome 3 restored the program of cellular senescence with or without the loss of telomerase activity and shortening of telomeres. Thus, chromosome 3 carries functionally distinct senescence genes, one of which is involved in the telomerase pathways. Radiation hybrid experiments with transfer of fragmented chromosomes showed the region to be a 3p14-21.

S-II-1

GENETIC ALTERATIONS AND DIAGNOSIS IN HUMAN BREAST CANCER.
Mitsuru EMI, Yoshio MIKI, Yusuke NAKAMURA (Inst. Geront.
Nippon Med. Sch., Kawasaki, Cancer Inst.,Tokyo, Inst. Med.
Sci., Univ.of Tokyo, Tokyo)

Predisposing mutations in a BRCA1 gene have been recently identified in germ-line of 17q-linked breast and ovarian cancer families. To understand the frequency, distribution and nature of BRCA1 mutations in Japanese breast cancer patients, we screened 1,000 unselected primary cancers for mutations in exon 11, which accounts for 61% of the entire BRCA1 coding sequence. Using a method based on multiplex single-strand conformational polymorphism (SSCP) analysis of multiple restriction fragments generated by restriction-enzyme digestion of amplified DNA, we identified eight mutations including four that we had previously found in a subset of these cases. All eight were germline mutations; four of them were non-sense mutations or small deletions resulting in premature stop codons, and the other four were missense mutations. The Japanese carriers of these mutant BRCA1 alleles had developed breast cancers at ages ranging from 45 to 62, five of them bilaterally. Taking into account the effect of various factors such as life-time risk of breast cancer, screening efficiency, and the region examined, we roughly estimate that 2-3 % of breast cancer in Japan is attributable to BRCA1 mutation and that 1 in 1,500-2,000 Japanese women carry a germline mutation in the BRCA1 gene.

S-II-2

HYPERLIPIDEMIA AND CORONARY HEART DISEASE.
Hideo HAMAGUCHI, Kimiko KOBAYASHI, and Tadao ARINAMI
(Dept. Med.Genet., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba)

The genetic etiology of coronary heart disease (CHD) is complex and heterogeneous. We are searching for the polymorphic loci responsible for multifactorial hypercholesterolemia. We are also evaluating relative risks for CHD in Japanese subjects with the polymorphic genes which have been reported to be associated with CHD in Caucasians. Analysis by QTL and affected sib-pair methods using 101 Japanese families with child sib-pairs collected through a hypercholesterolemic child suggested the presence of the locus responsible for the variation of serum cholesterol levels and hypercholesterolemia in the apoAII/CIII/AIV gene cluster region ($p=0.00007$ and $p=0.02$, respectively). On the other hand, odds ratio for the risk of CHD calculated based on case-control studies was 1.58 (95% CI, 1.0-2.4) in subjects with the apoE4 allele, 2.5 (1.6-4.1) in subjects with the apo(a) alleles of kringle 4 repeats less than 24, and 2.3 (1.5-3.7) in subjects with the methylenetetrahydrofolate reductase (MTHFR) TT genotype. The presence of a synergistic effect of the apoE4 allele and the MTHFR TT genotype on the risk of CHD was suggested. For the predictive genetic diagnosis of CHD, further extensive studies are required, except for familial hypercholesterolemia.

S-II-3

GENETIC STUDIES OF ALCOHOLISM. Taro MURAMATSU and Susumu HIGUCHI.
Department of Psychiatry, Kurihama National Hospital, Kanagawa, Japan.

Twin and adoption studies consistently show the involvement of genetic factors in alcoholism. Prevention of this common disease is, at least theoretically, simple: Drink sensibly or abstinence. Accordingly, search for genes related to alcoholism will be of great help in the future preventive activities.

1. Alcohol metabolizing enzymes: Ethanol is eliminated from the body by oxidation to acetaldehyde and then to acetate, reactions catalyzed by alcohol dehydrogenase (ADH2) and aldehyde dehydrogenase (ALDH2), respectively. The latter gene has a common mutation which leads to inactivation of the enzyme activity. Individuals who have this mutant allele, ALDH2*2, are prevented from becoming alcoholics because of acetaldehydemia and associated unpleasant symptoms after drinking. In Japan, approximately 10% of alcoholics have this allele, compared with approximately 50% of controls.

2. Dopamine system: Because its involvement in reward-seeking behavior, dysfunction of dopamine system could be the biological factor of alcoholism. Thus polymorphic genes of this system have been targets of association studies of alcoholism, with conflicting results, due mostly to the heterogeneity of the disease. One strategy to obtain a homogeneous group is to focus on alcoholics with ALDH2*2 allele, because these patients should have some strong factor(s) to overcome the negative risk factor. In fact, allele distributions of dopamine D4 receptor and dopamine transporter differed between this group and control.

S-II-4

A PROSPECT OF EARLY PREVENTION OF PREGNANCY-INDUCED
HYPERTENSION USING GENETIC AND ENVIRONMENTAL RISK FACTORS.
Gen KOBASHI (Dept. Public Health, Hokkaido Univ. School of Med., Sapporo)

Pregnancy-induced hypertension (PIH) occurs via an interaction of genetic and non-genetic factors. To elucidate synergism of predisposing genes and non-genetic factors in PIH, 62 cases and 93 controls were studied. A variant of angiotensinogen (AGT) gene (Met²³⁵→Thr) in the exon 2, and that of angiotensin type 1 receptor (A1R) gene (A¹¹⁶⁶→C) in 3'-untranslated region were analyzed by PCR and hybridization. Lifestyles were mail-questionnaired, 1-6 months after delivery. Fourteen factors out of 100 items were selected ($p < 0.1$) by univariate analysis. After a stepwise regression analysis, 5 factors; 'TT genotype of AGT gene', 'AC+CC genotype of A1R gene', 'BMI \geq 24 before pregnancy', 'low consumption of milk during pregnancy' and 'mentally hard during pregnancy', were screened. Compared with a reference group with none of the risks, those with the 5 major risk factors were 735.1 times more liable to PIH in a multiple logistic model. It may give a clue to establish an effective individualized prediction and prevention of PIH, by eliminating manageable risk factors in lifestyles, particularly among the inherited high-risk individuals. Social and ethical issues on notification, informed consent, framework for intervention, evaluation of the effectiveness are problems to be evaluated in order to establish a prevention program for PIH.

General Contributions

A-1

THE POSSIBLE RELATIONSHIP BETWEEN CONSTITUTIONAL CHROMOSOME ANOMALIES AND DEVELOPMENT OF ACQUIRED CYSTIC DISEASE OF THE KIDNEY (ACDK) AND PAPILLARY RENAL CELL CARCINOMA IN LONG-TERM HEMODIALYSIS PATIENTS. Mitsuaki A. YOSHIDA¹, Yukio KAGEYAMA², Hideyuki OGASAWARA³, Akihiro SHISHIKURA⁴, Hiroyuki OSHTMA² and Tatsuro IKEUCHI¹ (¹Dept. Cytogenet., Med. Res. Inst., ²Dept. Urol., Sch. Med., Tokyo Med. & Dent. Univ., Tokyo, ³Dept., Urol., Sch. Med., Kumamoto Univ., Kumamoto, ⁴Blood Transf. Ser., Jikei Univ. Sch. Med., Tokyo)

Acquired cystic disease of the kidney (ACDK) develops in long-term haemodialysis patients, and renal cell carcinoma (RCC), particularly, of papillary type, is frequently observed in ACDK patients. To understand the possible genetic mechanism involved in the pathological states, we performed chromosome analysis in ACDK and papillary RCC from hemodialysis patients. During the course of this study, chromosomes of blood lymphocyte cultures from these patients have also been studied, and 4 cases among 55 patients analyzed showed constitutional chromosome abnormalities; They include one case each of del(15q) in Prader-Willi syndrome, XYY male, and two reciprocal translocations, 7q;19q and Xp;10q. The incidence of such constitutional chromosome abnormalities in hemodialysis patients was extremely higher than that in the general population. Papillary RCCs from 5 hemodialysis patients have been chromosomally analyzed, and 2 of them showed various types of acquired chromosome abnormalities, such as 7- and 16-trisomies, partial trisomy of 10, inv(Y) and unbalanced translocation of 1q and 10p; there have been no structural anomalies possibly related to the above constitutional chromosome translocations. Further chromosome studies are needed in an increased number of hemodialysis patients.

A-2

EPIDEMIOLOGY OF GENITO-URINARY MALFORMATIONS ASSOCIATED WITH CHILDHOOD MALIGNANCIES IN JAPAN. Jun ABE, Masako TANIMURA, Ichiro MATSUI (Dept. Child Ecology, National Children's Med. Res. Ctr., Tokyo) and Noboru KOBAYASHI (National Children's Hospital, Tokyo)

Between 1962 and 1992, 29,685 children with malignant tumors were registered at the JCCR (Japan Children's Cancer Registry) at Tokyo. They consist of leukemia (40.4%), malignant lymphoma (8.2), brain tumor (8.8), neuroblastoma (12.0), retinoblastoma (7.7), germ cell tumor (7.1), Wilms tumor (3.9), hepatoblastoma (2.3), and the other tumors (9.7).

Among these tumors, Wilms tumor and germ cell tumor had the higher incidence of the congenital genito-urinary malformations compared with the other tumors. In Wilms tumor, hypospadias and cryptorchidism were seen in 3.0% and 4.9% of male patients, respectively ($p < 0.01$), and in germ cell tumor, cryptorchidism was seen in 1.4% of male patients ($p < 0.01$). The earlier age at the diagnosis of Wilms tumor was seen in patients with hypospadias ($p < 0.01$) and with cryptorchidism ($p < 0.05$) as reported in patients with AGR and Drash syndromes in which WT1 gene mutation has been found.

Wilms tumor was also associated with the higher incidence of renal-ureteral dysplasia such as fused kidney (0.4%) and duplicated kidney (0.2%). Besides Wilms tumor, neuroblastoma had an elevated incidence of hemi-hypoplastic kidney (0.1%) and duplicated kidney (0.1%) ($p < 0.01$). The association of renal-ureteral dysplasia with these two solid tumors and the absence of the relationship with early onset in these patients may suggest the other possible genes than WT1 gene that work in both carcinogenesis and organogenesis.

A-3

FAMILY HISTORY OF NEUROBLASTOMA

Masako TANIMURA, Ichiro MATSUI (Dept. Child Ecology, Natl. Children's Medical Research Center) and Noboru KOBAYASHI (Natl. Children's Hospital)

We reviewed family histories of 3808 neuroblastoma (NB) cases, diagnosed between 1969 and 1992, from among those entered in the Japan Children's Cancer Registry. Three siblings of NB probands in 3 families and 2 cousins in 2 families died of NB. In these families NB patients were diagnosed at 0-6 years old, suggesting that the NB mass screening for 6-month infants is somewhat effective for early detection of malignant-type NB.

The rate of probands with a history of an aunt's uterine cancer in NB probands (0.45%) was significantly higher than in other childhood cancer probands (0.21%). Out of 22 families of NB probands having a history of aunt's uterine cancer, other type of cancer occurred in aunt's sibs in 3 families and abortions or still births occurred in proband's sibs in 3 families. The uterine cancer incidence in grandmothers of NB probands was also significantly higher than in those of other childhood cancer probands. These facts imply that there is a genetic factor associated both with NB in childhood and uterus cancer in adults.

A-4

CORRELATION OF LOSS OF HETEROZYGOSITY WITH CLINICOPATHOLOGICAL PARAMETERS IN PRIMARY BREAST CANCER. ^{1,2}Satoshi MATSUMOTO, ¹Mitsuru EMI, ²Masahiko ONDA, ³Masataka YOSHIMOTO, ³Fujio KASUMI, ⁴Yusuke NAKAMURA (¹Dept. Mol. Biol., Inst. Gerontol., Nippon Med. Sch., Kawasaki., ²1st. Dept. Surg., Nippon Med. Sch., Tokyo., ³Dept. Surg., Cancer Inst. Hosp., Tokyo., ⁴Labo. Mol. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

In order to investigate the role of loss of heterozygosity (LOH) during tumor development and/or progression, we looked for correlations between various clinicopathological parameters of breast cancer and LOH at chromosomal regions, 1p, 3p, 8p, 9q, 11p, 16q, 17p, 17q and 22q, where we have been detected frequent losses in primary tumors. In the present study, we examined 152 primary breast cancers for LOH at 18 regions on the 9 different chromosomal arms using 24 highly informative microsatellite markers. Our results suggest the following below. 1) LOH at 16q22-24 and 17q21 might be early events in breast carcinogenesis. 2) LOH at 3p14-21, 11p15.5, 13q12-14 and 17q21 could be associated with the loss of hormone dependency in progression of primary breast cancer. 3) LOH at 1p22-31 and 17p13.3 could have a significant role during the regional lymphnode metastasis.

A-5

HUMAN NEDD-4 AS A FAMILY MEMBER OF UBIQUITIN LIGASES :

Tadashi ANAN^{1,2}, Yoichi NAGATA¹, Yoshiomi HONDA¹, Yoriyoshi KIMURA¹, Akira KUWANO³, Nobuo NOMURA⁴, Ichiro MATSUDA², Hideyuki SAYA¹, Mitsuyoshi NAKAO¹ (¹Dept. Tumor Genet. Biol., ²Dept. Pediatr. Kumamoto Univ. Kumamoto., ³Dept. Hygiene, Ehime Univ. Matsuyama., ⁴Kazusa DNA Research Institute. Kisarazu)

Ubiquitin-proteasome proteolytic pathway is involved in a number of cellular physiology. In this system, a ubiquitin ligase, or E3 enzyme, is a major component which recognizes a specific substrate protein for targeted degradation. E6-associated protein (E6-AP) has been recently found to be a member of the E3 enzyme family that is characterized by a conserved carboxy-terminal sequence, termed *hect*-domain. Using human EST data bases, we identified five uncharacterized genes of this family. By FISH and radiation hybrid panel, these genes were mapped to subchromosomal locations as follows; E6-AP (15q12-13), NEDD-4 (15q22; D15S209), D28476 (2q35-36; D2S396), D13635 (7q36; FB16B4), and D25215 (4q21-22; WI-6336). NEDD-4 gene was mapped close to the translocation breakpoint of acute promyelocytic leukemia. Interestingly, NEDD-4 was highly conserved among species from yeast to human, suggesting its significant function on basic biomodulation. Northern blot analysis showed that NEDD-4 mRNA was much higher expressed in lung carcinoma A549 and chronic myelogenous leukemia K562 than in other cancer cell lines tested. The rest of the members, except D25215, were ubiquitously expressed in all cells. In immunoblot analysis, human NEDD-4 protein was down-regulated during neural differentiation in BDNF-treated neuroblastoma cells. Collectively, our data suggest that NEDD-4 protein may function as a ubiquitin ligase closely related to development, differentiation and tumorigenesis.

A-6

FUNCTIONAL ANALYSIS OF TUMOR SUPPRESSOR P53 PROTEIN REGULATED BY UBIQUITIN-PROTEASOME SYSTEM. Yoichi NAGATA, Tadashi ANAN, Yoshiomi HONDA, Reiji KIMURA, Hideyuki SAYA, Mitsuyoshi NAKAO (Dept. of Tumor Genet. and Biol., Kumamoto Univ., Kumamoto) Motomi NAKATA, Naoki SHINBARA (Sumitomo Electrical Industry, Yokohama) Keiji TANAKA (The Tokyo Metropolitan Inst. of Med. Sci., Tokyo) Satoshi OMURA (Kitazato Inst., Tokyo)

The tumor suppressor p53 protein is most important for cell regulation such as cell cycle and apoptosis. In HPV-infected cells including HeLa cells, a viral oncoprotein E6 and a cellular E6-associated protein (E6AP) function together as a ubiquitin ligase targeting for p53. The complex plays a major role in the tumorigenesis by rapid turnover of p53. However, the mechanism of p53 degradation in normal cells remains unclear. 1) Using proteasome inhibitors, p53 protein was significantly accumulated in primary fibroblasts as well as HeLa cells in vivo. 2) Recombinant p53 proteins destined for wild- or mutant(273His)-type were analysed in an appropriate buffer and rabbit reticulocyte lysates in vitro. Much slower degradation of mutant-p53 was found compared with the case of wild-p53. Interestingly, both-types of p53 were multi-ubiquitinated in a similar manner. 3) In fibroblasts infected with p53-expressing adenovirus, wild-p53 protein was co-immunoprecipitated with anti-E6AP antibody. In summary, E6AP may also work in the p53 ubiquitination in normal cells adding to E6-dependent case. Ubiquitinated mutant-p53 becomes resistant to degradation, suggesting that mutant-p53 is hardly recognized and/or degraded by proteasome due to unknown mechanism.

A-7

DETECTION OF p53 MUTATION IN HUMAN AND RAT TUMOR CELLS BY YEAST FUNCTIONAL ASSAY. Daichi NAKATA¹, Hidefumi TONOKI¹, Haruhiko KASHIWAZAKI¹, Yi BA¹, Jun-ichi HAMADA¹, Masuo HOSOKAWA², Mitsuhiro TADA³ and Tetsuya MORIUCHI¹ (¹Lab.Cell Biol., ²Lab. Pathol. Cancer Inst. and ³Dept. Neurosurgery Hokkaido Univ. School of Med., Sapporo)

Yeast functional assay is a simple and sensitive screening method for detection of p53 mutation in human tumor tissue. The RT-PCR products are directly assayed according to its DNA binding capacity in yeast. Relative population of cells carrying mutant p53 allele is ascertained by counting red colonies. We analyzed the sensitivity and the clinical application of this method. Mutant p53 alleles are detectable from samples containing at least 6% of mutant p53 cDNA. It is disclosed that more than 20% of red colonies suggests the existence of clonal expansion of cells carrying mutant p53 allele. We reconstructed this method to establish a yeast assay for rat p53 gene. By this method, several p53 mutations were newly identified in rat tumor cell lines. Hepatocellular carcinoma and cholangiofibrosis tissues in LEC rat liver were then assayed with this method. Ratios of red colony were significantly elevated in these tissues, but were not large enough to suggest the existence of clonal p53 mutation. This result suggests that p53 mutation is not a primary event for the development of hepatocellular carcinoma in LEC rat and that increased red colonies may indicate a possible genomic instability in LEC rat liver. In conclusion, the yeast functional assay is sensitive and useful method for the detection of clinical mutation. This assay for rat p53 gene provides a new monitor system of p53 status in various experiment.

A-8

Isolation and Characterization of chromosome specific gene, that contains a variable number of tandem repeats (VNTR) site. Kumiko KOYAMA, Mieko MATUSHIMA and Yusuke NAKAMURA(Lab. Molecular Medicine, Ins. Med. Sci., Univ. Tokyo)

Variable number of tandem repeat (VNTR) markers, which tend to be highly polymorphic, have contributed to many studies in positional cloning and forensic medicine. In addition to their usefulness as genetic markers to distinguish between alleles, VNTR loci may have a biological role, have recently indicated that the number of repeats within the VNTR that lies in the 5' region of the insulin gene is likely to be associated with susceptibility to insulin-dependent diabetes mellitus.

Now we have isolated and characterized a number of novel human genes, that contains VNTR sequences. Furthermore, we tried functional assay to these genes.

A-9

SELECTION OF CHROMOSOME 12 SPECIFIC ALPHA SATELLITE DNA USING NYLON MEMBRANE. Takazumi KOZAKI, Yasunobu YOKOYAMA, Tadashi KAJII (Genetic Research Division, Center for Molecular Biology and Cytogenetics, SRL, Inc.)

We have isolated 1.4kb α -satellite DNA of chromosome 12 using a combination of microdissection, PCR amplification, hybridization of the amplified products with larger DNA fragments, and selection of the hybridized segments with nylon membrane (Satish Parimoo et al., 1995).

The centromeric region of chromosome 12 was microdissected, a 171 bp segment of microdissection products was amplified using α -satellite consensus primers, and the amplified DNAs were cloned. Southern blot analysis of Hind III-digested human genomic DNAs revealed a 1.4kb band with the 171 bp cloned DNAs as a probe (Looijenga L.H.J. et al., 1991). DNAs were extracted from the band, PCR amplified with M13 primers, and selected with hybridization to the 171 bp DNAs immobilized onto a nylon membrane. The selection process was repeated twice. The selected products were checked with chromosomal FISH for their specificity, and cloned in pBluescript II. Cloning efficiency was one in 27 clones with the membrane selection process, while it was 0 in 192 clones without the process.

A-10

MICRODISSECTION-MEDIATED ISOLATION OF CHROMOSOMAL REGION-SPECIFIC CDNAS. Yasunobu YOKOYAMA, Takazumi KOZAKI (Center for Molecular Biology and Cytogenetics, SRL, Inc., Tokyo) and Keiko OHSUGI, Norio SAKURAGAWA (National Institute of Neuroscience, NCNP., Tokyo)

K562 is a cell line with two acrocentric marker chromosomes each with abnormally banded regions (ABRs), derived from Ph-positive CML. Using reverse and forward chromosome painting FISH analysis, we found that 9q34, 13q31, and 22q11 regions co-amplified in the ABRs. Utilizing the ABRs as target DNA of cDNA selection, we established a simple procedure for the isolation of chromosomal region-specific cDNAs.

First strand cDNAs were synthesized from fetal brain mRNA with an oligo-(dT)17 primer. With the 1st strand cDNAs as templates, short-fragment cDNAs (sf-cDNAs) were synthesized using modified Degenerate Oligonucleotide Primed Shuttle Polymerase Chain Reaction (DOP-Shuttle-PCR) (Cytogenet Cell Genet 71:32-36, 1995). The sf-cDNAs were hybridized onto RNase A-treated metaphases from K562, and their ABRs were microdissected and reamplified with DOP-Shuttle-PCR primer II. Ladder amplification products, ranging from 150 to 400 bp were observed by gel electrophoresis. The reamplified sf-cDNAs were cloned into a pBluescript vector. Randomly chosen 20 clones were sequenced and classified into 8 groups. Three out of the 8 clones had been mapped at 22q11, whereas the other 5 were novel cDNAs. Quantitative Southern blot analysis indicated that 7 out of the 8 clones (87.5%) to be derived from the co-amplified regions.

A-11

A NOVEL 4.7-kb TANDEM REPETITIVE SEQUENCE "RS447": HIGHLY POLYMORPHIC IN JAPANESE POPULATION. Yoichi GONDO^{1,2}, Takeya OKADA¹, Shinji HADANO^{1,2}, Jun GOTO³, Ichiro KANAZAWA³ and Joh-E IKEDA^{1,2} (¹Inst. Med. Sci., Tokai Univ.; ²JST/Canada Int'l Res. Exchange Program "Neurogenes"; ³Univ. of Tokyo Sch. Med.)

While positional-cloning of the Huntington's disease (HD) gene at the Human 4p region, a novel tandem repetitive sequence RS447, a unit size of which was 4746 bp was identified (Kogi et al., in preparation). Pulsed-field gel electrophoresis revealed that the copy number of the RS447 tandem repeats was highly polymorphic ranging between 12 and 90 in Japanese population. Each allele has been found to be transmitted to offspring based upon the Mendelian's genetics. There was an open reading frame of 1629 bp within which the consensus donor as well as acceptor exon-intron boundary sequences existed. RS447 was a novel sequence by database search but conserved in mammals. RS447, therefore, might be expressed with a biological function(s). We call the RS447 tandem repetitive sequence "megasatellite".

A-12

STRUCTURAL ORGANIZATION AND CANDIDATE GENE ANALYSIS OF X - ARRESTIN. Hitoshi SAKUMA, Toshihiro YAJIMA, (Dept. of Ophth., Juntendo Univ., Tokyo) Tomomi HIGASHIDE, (Dept. of Ophth., Kanazawa Univ., Kanazawa) Akira MURAKAMI, (Dept. of Ophth., Nat. Defense Medical College, Tokorozawa) Margaret McLAREN and George INANA (Bascom Palmer Eye Institute, Univ. of Miami School of Medicine, Florida, U.S.A.)

X - arrestin is a newly isolated human retinal arrestin mapping to X - chromosome. X - arrestin is enriched in retina and its sequence is homologous to Arrestin (S - Ag) and β - arrestin. The role of S - Ag is thought to quench the activated phototransduction cascade by binding to phosphorylated rhodopsin. Recently, 1 - bp deletion of the arrestin gene was found in some patients with Oguchi disease. Otherwise, our immunohistochemical study by using anti - peptide antibody to human X - arrestin showed that X - arrestin is expressed intensely in cone photoreceptors in human retina. Although the precise function of X - arrestin is unknown, these results suggest that X - arrestin might be a cause of retinal dysfunction and it may be useful as a candidate gene for X - linked retinal diseases. To obtain information of gene structure, X - arrestin genes were isolated by screening human genome libraries using cDNA as a probe and analyzed by sequencing. The X - arrestin gene has 17 exons and is approx. 20 kbp long. Its structure has similarity to S - Ag already reported. 96 X - linked retinal disease patients were screened for mutation by PCR - DGGE analysis. Some types of mobility shift were observed, but no exon mutation was found except silent mutation at exon 3 and polymorphism at exon 12. Further analysis of X - arrestin gene will contribute for better understanding of cone photoreceptor visual transduction system.

A-13

A MUTATION OF *CHM* GENE IN A JAPANESE CHOROIDEREMIA PATIENT AND HIS MOTHER. Takuro FUJIMAKI, Yoshihiro HOTTA, Keiko FUJIKI, Mutsuko HAYAKAWA, Rie DOI, Nao KOHNO, Misako TAKEDA, Atsushi KANAI (Dept. Ophthalmol., Juntendo Univ., School of Med., Tokyo)

Choroideremia is a X-linked chorioretinal dystrophy in which clinical feature is similar to typical retinitis pigmentosa. Choroideremia (*CHM*) gene was recently isolated and characterized. Although *CHM* gene defects were reported in patients with choroideremia in Europe, no report was recognized in the other countries. We analyzed the *CHM* gene in Japanese patients with choroideremia and found a family with a mutation in *CHM* gene. DNAs were isolated from peripheral blood in 4 patients from 4 Japanese families with choroideremia. Exons 11,12,13 and 14 were amplified by PCR using primers (van den Hurk et al, Am J Hum Genet,1992) encompassing these exons. The PCR products were screened by SSCP (single strand conformation polymorphism) and directly sequenced. Mutation from A to CC in the nucleotide position 1608 of *CHM* gene was detected in a patient. His mother was heterozygote of wild and mutant type of *CHM* gene. Other 3 patients from 3 Japanese families have no mutation in exons 11,12,13 and 14.

A-14

ANALYSES OF PERIPHERIN/*RDS* GENE IN SEVERAL RETINAL DEGENERATIONS. Keiko FUJIKI, Yoshihiro HOTTA, Mutsuko HAYAKAWA, Rie DOI, Nao KOHNO, Misako TAKEDA, Atsushi KANAI (Dept. Ophthalmol., Juntendo Univ., School of Med., Tokyo) Yasushi ISASHIKI, Norio OHBA ('Center for Chronic Viral Diseases and Dept. Ophthalmol., Fac. of Med., Kagoshima Univ., Kagoshima)

To detect a peripherin/*RDS* gene defect in patients with several retinal degenerations, genomic DNAs were extracted from peripheral blood of 77 patients with autosomal dominant retinitis pigmentosa (40), cone rod dystrophy (11), vitelliform macular degeneration (4), macular degeneration (3), rod cone dystrophy (2), cone dystrophy (2), fundus albipunctatus (2), fundus flavimaculatus (2) and retinitis pigmentosa with macular degeneration (11). Three exons of the peripherin/*RDS* gene were amplified to DNA fragments of approximately 200 base pairs by polymerase chain reaction (PCR) method and analyzed by single strand conformation polymorphism (SSCP). The DNA fragments suspected of any variation were directly sequenced.

Seven point mutations were detected. Among them Glu304Gln and Gly338Asp mutations have substituted amino acid. Nevertheless nobody was co-segregated with the disease, and also these mutations were found in normal controls with high frequencies in which the rate of the variation was 0.195 ± 0.028 in codon 304 and 0.194 ± 0.038 in codon 338. Further studies for another candidate genes are required.

A-15

MISSENSE MUTATION IN THE C-TERMINAL PART OF THE PAX6 PAIRED DOMAIN IN A FAMILY WITH ISOLATED FOVEAL HYPOPLASIA. Noriyuki AZUMA¹, Sachiko NISHINA¹, Hiroko YANAGISAWA², Torayuki OKUYAMA² & Masao YAMADA². (¹ Dept. of Ophthalmology, National Children's Hospital, ² National Children's Med. Res. Ctr., 3-35-31, Taishido, Setagaya, Tokyo 154).

The fovea is an excavated area of the retina at the posterior end of the visual axis that facilitates visual acuity. Whereas foveal hypoplasia with decreased visual acuity and congenital nystagmus is a common feature of recognized ocular disorders such as albinism and aniridia, isolated foveal hypoplasia which is not associated with other known ocular abnormalities is rare and sporadic. Although a single family with autosomal dominant isolated foveal hypoplasia has been previously reported (MIM 136520), the molecular defect segregating in this kindred has not been identified. We have now ascertained a second family with isolated foveal hypoplasia and detected a missense mutation in the PAX6 gene. The mutation occurred in the C-terminal part of the paired domain, which is the first mutation identified in this region in any member of the PAX gene family (Azuma et al. *Nature Genet.*, 13, 13, 141-142, 1996). Although PAX6 is well known to be involved in eye morphogenesis as demonstrated in aniridia and Peter's anomaly, the phenotype-genotype correlation in this family raises an interesting hypothesis regarding the function of Pax proteins.

A-16

MULTIPLE ALLELES RESPONSIBLE FOR A₂ AND A₂B PHENOTYPES

Kenichi OGASAWARA¹, Ryuichi YABE¹, Kenichi NAKATA¹, Michiko TAKENAKA¹, Yuiji TAKAHASHI¹, Makoto UCHIKAWA² and Katsushi TOKUNAGA^{2,3} (1. Japanese Red Cross Tokyo Metropolitan Blood Center, 2. Japanese Red Cross Central Blood Center, 3. Department of Human Genetics, Graduate School of International Health, University of Tokyo).

In some populations, including Japanese, the frequency of A₂B phenotype is significantly higher than that expected based on the A₂ phenotype frequency. To explain the high A₂B phenotype frequency, we have examined relative frequencies of the presence of ABO alleles in selected individuals with A₁, A₁B, A₂ and A₂B phenotypes. Using PCR-SSCP and nucleotide sequence analyses, we identified five different alleles, *A105, *A106, *A107, *A111 and *R101, in A₂- and A₂B-positive samples. In A₂-positive samples, *A106 and *A107 were common with frequencies of 48% and 41%, respectively. In A₂B-positive samples, both alleles were uncommon and *R101 was predominant (41%). When 401 A₁ samples were examined, 1% of them were found to possess *R101, but neither *A106 nor *A107 was detected. These results indicate that *A106 and *A107 encode a catalytically weak transferase. *R101 is presumably expressed as A₁ in *R101/*O individuals, but as A₂ in *R101/*B individuals, thus giving rise to high A₂B phenotype frequency.

A-17

DE NOVO RECOMBINATION CASE AT THE ABO LOCUS AND OCCURRENCE OF RECOMBINANTS IN A RANDOM POPULATION. Hiroko TSUJI, Misa IWATA, Kiyoshi MATSUI, Akiyoshi TAMURA, Tokiko MIYAZAKI, Koichi SUZUKI, (Dept. Legal Medicine, Osaka Medical College, Takatsuki)

In a paternity case where parentage between the father and child was excluded only at the ABO locus (mother: B, child: A, father: O), a hybrid allele was found to be involved in the discrepancy. Sequencing of PCR products spanning exon 6 to 7 of the ABO gene for the three showed that one allele of the child had a hybrid nature, consisting of the B and O1 sequences in exon 6 and 7, respectively (abbreviated as b-o1). Survey of a Japanese random population for such hybrid alleles by using SSCP analysis demonstrated relatively frequent occurrence of them in a Japanese population. Sequencing showed that these were b-b/olv, o1-olv, olv-a1 (or -o1) with their sequences changing within exon 7, intron VI, and intron VI-exon 7 boundary, respectively. This result suggested that there are recombination hotspots in the gene region.

A-18

THE GENE ANALYSIS OF A JAPANESE PROPOSITUS WITH D-- PHENOTYPE. Hiroshi OKUDA, Masaki KAWANO, Eiji KAJII (Dept. Legal medicine and Human genetics, Jichi Medical School, Tochigi) and Tomoko ISHIDA, Shosaku NOMURA, Shiro FUKUHARA (Dept. Transfusion Service, Kansai Medical College, Osaka)

Recent study on Rh blood group system is extremely progressed. The two genes theory (*RHCE* gene, *RHD* gene), which was previously suggested, is acceptable by recent molecular studies. We analysed a Japanese D-- propositus and parents with molecular genetics methods based on new knowledges. The gene analyses of D-- propositus showed a gross deletion of *RHCE* gene and a new rearrangement of *RHCE*; *CE-D-CE* hybrid gene. It was demonstrated that the hybrid gene was composed of exon 1 from the *RHCE* gene, followed by exons 3 to 7 from the *RHD* gene and exons 8 to 10 from the *RHCE* gene. It was undetectable which exon 2 of the *RHD* or *RHCE* gene is contained in the *CE-D-CE* gene. The D-- gene complex from paternal side comprised the *RHD* and *CE-D-CE* gene. On the other hand, that complex from the maternal side seemed to be only the *RHD* gene. The Rh phenotype of mother was CcDEe. There are two hypotheses for explaining this discrepant event in mother. The other gene complex may be composed of two or three genes; two *RHCE* and/or one *RHD* genes. Otherwise, the D-- gene complex might be produced at the stage of gametogenesis. The further study - the analysis of intron which is the hybrid site - shall be carried out.

A-19

ANALYSIS OF RH RELATED cDNAs IN A PROPOSITUS OF REGULATOR TYPE R_hNULL SYNDROME. Sadahiko IWAMOTO, Masaki KAWANO, Hiroshi OKUDA, Eiji KAJII (Dept. Legal Med. and Human Genet., Jichi Medical School, Tochigi)

The erythrocytes of R_hNULL phenotype lack all Rh blood group antigens. R_hNULL may arise from two genetic backgrounds; R_hNULL regulator type, occurs by an autosomal suppressor gene that unlinks to RH gene, R_hNULL amorph type, occurs by homozygosity of a silent allele of RH locuses. R_hNULL cells lack not only Rh antigens, but LW antigen and Rh50 glycoprotein. Severe reduction of CD47 glycoprotein and glycophorin B have also been reported. Among them, the Rh50 glycoprotein was proposed as a most plausible candidate for a suppressor gene of regulator type R_hNULL. We analyzed the Rh50 cDNA of a propositus with R_hNULL phenotype. RT-PCR analysis from reticulocytes RNA revealed that the Rh50 cDNA deleted 122bp fragment from nt. 902 to 1052, despite any mutations were not observed in RhD and CE cDNAs. The 122bp deletion also gave rise to frame-shift and resulted in short and abnormal Rh50 glycoprotein. Then, the Rh50 glycoprotein was regarded as a most essential factor for the expression of Rh antigen complexes.

A-20

A NEW DETECTION METHOD OF POINT MUTATION BASED ON PCR-PRIMER INTRODUCED RESTRICTION ANALYSIS(PCR-PIRA)

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(Dept. Biochem.¹, Legal Med.², Kawasaki Med. School)

A new detection method of PCR-PIRA using a specific mismatched primer was introduced to detect carriers in family members of the patient with silent type BChE variant simply and rapidly. The specimens from two families with different BChE variant was used. At the first case(BChEY128C), a new Tsp45 I restriction enzyme site was introduced into the PCR product from the aberrant allele and at the second case (BChEA199), a new Pst I site was created into the PCR product from the normal allele. The digest obtained from the family members by treatment of two enzymes, Tsp45 I and Pst I, were separated by capillary gel electrophoresis(CGE). In a total of 14 members containing the propositus in two families, four members were homozygote and seven were heterozygote. These results demonstrate that the PCR-PIRA method is a recommendable procedure to family analysis.

A-21

MOLECULAR ANALYSIS OF FAMILIAL HYPOTRANSFERRINEMIA

Midori Asada SENJU, Toyoki MAEDA, Yoshinao WADA*, Akira HAYASHI* and Tomokazu SUZUKI (Dept. Clin. Genet., Med. Inst. of Bioregulation, Kyushu Univ., Beppu and *Osaka Medical Center and Research Institute for Maternal and Child Health)

We previously reported a clinical course of a patient with familial hypotransferrinemia for 20 years from seven years of age, who was suffered from severe anemia and growth retardation deduced from the trace level of serum TF (transferrin). The isoelectric focusing study revealed that his father's serum TF had an abnormal isoelectric point.

Nucleotide sequence analysis of the TF gene revealed that the propositus was a compound heterozygote of a maternal null allele concomitant with a synonymous mutation, a C to T transition at the position 760, and a paternal mutant allele with a G to A transition at the position 1210 changing glutamic acid to lysine at the amino acid residue 404. The paternal mutation was not thought to be a polymorphism, because this mutation was not found among 100 people analyzed with PCR-RFLP. Further study is necessary to delineate the maternal unknown null mutation.

A-22

MOLECULAR ANALYSIS OF HEREDITARY METHEMOGLOBINEMIA. Jun-ichi MANABE^{1,2}, Roopen ARYA³, Hideki SUMIMOTO⁴, Masataka MIYAZAKI⁵, Toshitsugu YUBISUI⁶, D. Mark LAYTON³, Yasuyuki FUKUMAKI¹ (¹Inst. Genet. Inf., Kyushu Univ., Fukuoka, ²Central Lab., CRC, Inc., Fukuoka, ³Dept. Haematol. Med., King's College School of Medicine and Dentistry, London, UK., ⁴Dept. Biochem, Kyushu Univ., Fukuoka, ⁵Gunma Children's Medical Center, Gunma, ⁶Dept. Biol., Kochi Univ., Kochi)

Hereditary methemoglobinemia is an autosomal recessive disorder characterized by NADH-cytochrome b5 reductase (b5R) deficiency. This disorder is classified into two types: an erythrocyte (type I) and a generalized (type II) types. We analyzed the b5R genes of a patient with type II from a white U. K. family using PCR-related techniques, and found that the patient was a compound heterozygote for two novel mutations, a nonsense mutation at codon 42 (TAC-TAA) and a missense mutation at codon 95 (CCC-Pro-CAC; His). From the allele with the nonsense mutation, the product without the catalytic portion of the enzyme is generated. To characterize the effect of the missense mutation on the enzyme function, we compared GST-fused b5R with the GST-fused mutant enzyme expressed in *Escherichia coli*. The mutant enzyme exhibited less catalytic activity, less thermostability and more susceptibility to trypsin than did the normal counterpart. The absorption spectrum of the mutant enzyme in the visual region differed from that of the wild type. These results suggested that this amino acid substitution within the FAD-binding domain of the enzyme influences both a secondary structure and a catalytic activity of the enzyme. One Japanese case of type II was also investigated. A novel nonsense mutation (GAG;Glu-TAG;Stop) was detected in the codon 255 of one allele, however no mutation is observed in another allele. A Greek-Cyprus case with type II, in which no obvious mutation is associated with the b5R gene, was presented. Possible molecular basis of such cases was discussed.

A-23

MOLECULAR ANALYSIS OF β -THALASSEMIA IN MALDIVES AND JAPAN. Takahito INOUE¹, Naila FIRDOUS², Hiroyasu FURUJUMI¹, Rie SEMURA^{1,3}, Hideo OHTA¹, Suthat FUCHAROEN⁴, Yasuyuki FUKUMAKI¹ (1)Ins.Gen.Info., Kyushu Univ., Fukuoka, (2)Soc.Health.Edu., Republic of Maldives, (3)C.R.C., Fukuoka, (4)Mahidol Univ., Sch. Med., Thailand)

β -thalassemia is a group of disorders characterized by impaired expression of the β -globin gene. It has a worldwide distribution with particularly high frequencies in the Mediterranean, Indian and Southeast Asian. In malaria endemic regions, the malaria hypothesis is a plausible explanation for the high prevalence of this disorder. To compare the molecular basis of the β -thalassemia in between malaria endemic and non-endemic regions and to trace the origin and spread of mutations in Asian populations, we analyzed β -thalassemia mutations and determined the haplotypes and frameworks in 45 Japanese β -thalassemia patients and 50 Maldivian patients using PCR-related techniques. The following results were obtained. 1) In the Maldivian population, five different mutations were detected. These are IVS1#5(G-C), Codon30(G-C), IVS1#1(G-A), β^E and Codon41/42(-4bp). In the Japanese population, 13 different mutations were detected. These are IVS2#654(C-T), Codon90(G-T), IVS2#1(G-A), -31ATA(A-G), Codon41/42(-4bp), Codon15(-T), Codon54(+G), Codon110(T-C), Codon123(-A), Codon127/128(-AGG), Codon121(G-T), IVS2#848(C-G) and Initiation codon(A-G). 2) Analysis of β -globin gene haplotypes and frameworks suggested that most common mutation, IVS1#5(G-C) in Maldivian β -thalassemia genes was derived from the Indian population, and Codon41/42(-4bp) mutation shown by both populations has different origin. 3) In the Japanese population, four dominant mutations were detected. The presence of such mutations in the Japanese population may be explained by a fact that Japan is not malaria endemic and there is a loss of heterozygous advantages against malaria infection in such mutations.

A-24

HEMIZYGOUS HAPTOGLOBIN GENE DELETION IS A CAUSE OF FAMILIAL ANHAPTOGLOBINEMIA. Yoshiro KODA, Mikiko SOEJIMA, Hiroshi KIMURA (Dept. Legal Med., Kurume Univ. School of Med., Fukuoka)

We have found haptoglobin (Hp) gene deletion (Hp^{del}) during the study of two families of anhaptoalbuminemia. Southern blot and DNA sequence analyses have indicated that the gene deletion have been included in the promoter region of the Hp gene, the α - and β -chain of Hp gene and the α -chain of haptoglobin-related (Hpr) gene but not the β -chain of Hpr gene. All 6 individuals tested for Hp^2/Hp^{del} genotype showed extremely low serum level of Hp (0.006-0.12 mg/ml) as compared with the average value (1.69 mg/ml) obtained from 95 healthy individuals. While we have found only one hemizygous individuals for Hp^1/Hp^{del} genotype, the plasma Hp in this individual is about half level of that in healthy individuals (0.5 mg/ml). The results suggested that the Hp^2 but not the Hp^1 appeared to be gene dosage-sensitive when the Hp^2 was associated with the Hp^{del} , and that the hemizygote for Hp^2/Hp^{del} was the probable cause for the anhaptoalbuminemia.

A-25

GENETIC POLYMORPHISMS OF CYP2C19 AND CYP2C9 GENES IN JAPANESE AND CAUCASIAN POPULATIONS. Kiyoshi INOUE¹, Hiroshi YAMAZAKI¹, Johji INAZAWA², Tatsuo ABE² and Tsutomu SHIMADA¹ (¹Osaka Pref. Inst. Public Health, Osaka, ²Dept. Hygiene, Kyoto Pref. Univ. Med., Kyoto)

We investigated genetic polymorphisms in the CYP2C19 and 2C9 genes in Japanese and Caucasian populations. Two defects in the CYP2C19 gene, a single-base pair mutation in exon 5 (CYP2C19_{m1}) and in exon 4 (CYP2C19_{m2}), have been reported in poor metabolizer phenotypes. Of 39 Japanese subjects tested, four were homozygous for the CYP2C19_{m1} mutation, one homozygous for the CYP2C19_{m2} mutation, and two heterozygous for CYP2C19_{m1}/CYP2C19_{m2} mutation. In contrast, only one subject was found to be homozygous for the CYP2C19_{m1} mutation in 45 Caucasian tested. Four variants in the CYP2C9 gene have been represented at Arg₁₄₄/Cys, Tyr₃₅₈/Cys, Ile₃₅₉/Leu, and Gly₄₁₇/Asp. Allele frequencies for Japanese subjects were determined to be 0.04 for Leu₃₅₉. Caucasian subjects showed frequencies for the Cys₁₄₄ and the Leu₃₅₉ alleles of 0.11 and 0.03, respectively. Our results indicate that the principle CYP2C9 allele is Arg₁₄₄Tyr₃₅₈Ile₃₅₉Gly₄₁₇ (frequencies of 0.96 and 0.86 in Japanese and Caucasian, respectively). We also found that there are marked interracial differences in the frequencies of polymorphisms in the CYP2C19 and 2C9.

A-26

ANALYSIS OF HIGHLY HOMOLOGOUS GENOMIC CLONES TO NITRIC OXIDE SYNTHASE II. Akira HATA¹, Akira KUWANO², Ituro INOUE³, Kiyotaro KONDO¹, and Jean-Marc LALOUEL³ (¹Dept. of Public Health, Hokkaido University School of Medicine, Sapporo, ²Dept. of Hygiene, Ehime University School of Medicine, Ehime, ³Howard Hughes Medical Institute, University of Utah, Salt Lake City, USA)

Nitric Oxide synthase (NOS) are a family of enzymes responsible for the synthesis of nitric oxide from L-arginine and molecular oxygen. So far, three NOS enzymes (I, II, III) and their genes have been identified and cloned. Expression of NOS II, unlike I and III, is induced by signals such as cytokines, and is independent of elevated Ca²⁺. For the purpose of isolating and characterizing genetic markers of human NOS II, we screened P1 library using PCR product from exon 25 to 26. Eleven clones were isolated, of these, two were NOS II, remaining four and five clones were revealed NOS2B and NOS2C reported by Bloch et al. (1995). In this study, we localized the loci of NOS2B and NOS2C to 17p11.2 and 17q11.2 by FISH method. Sequencing analysis of NOS2B (exon 21- 25) and NOS2C (exon 23-25) revealed that both genes were about 91% homologous to NOS II cDNA. If these genes are expressed, they must have important roles.

A-27

GENETIC SCREENING OF HYPERLIPIDEMIA TYPE IIa: IDENTIFICATION OF GENETIC MUTATIONS OF THE LDL RECEPTOR GENE IN 14 CASES. Tsunenori HIRAYAMA, Mitsuru EMI, Emi YAMAKI (Dept. Molecular Biology, Institute of Gerontology, Nippon Medical School, Kawasaki), Masahiro TSUJI (Internal Medicine, Hokkaido Social Security Central Hospital, Sapporo), and Akira HATA (Dept. Public Health, Hokkaido Univ., Sapporo)

Mutation of the low density lipoprotein (LDL) receptor gene cause familial hypercholesterolemia (FH), a genetic entity that manifests type IIa hyperlipidemia. We examined 55 cases of type IIa hyperlipidemia patients for mutation of the LDL receptor gene by PCR-SSCP and direct sequencing analysis. We identified 9 genetic mutations in 14 cases.: the -84 G→A mutation in exon 1, a nonsense mutation in exon 3 (the C74X mutation), a missense mutation in exon 8 (the C337Y mutation), a splice site mutation in intron 11 (the 1705+1 G→T mutation), a missense mutation in exon 12 (the W556C mutation), a 1-bp deletion of nucleotide position 1963 in exon 13, a nonsense mutation in exon 17 (the K790X mutation), and a missense mutation in exon 18 (the A829V mutation). The 3 cases with the C74X mutation were sisters, and the 2 cases with the W556C mutation were mother and son. Other mutations were found in unrelated individuals. Though one of the 9 mutations (K790X) was described previously, other 8 belong to new kind of mutations of the LDL receptor gene.

A-28

MUTATIONS OF THE LDL RECEPTOR GENE IN THREE FAMILIAL HYPERCHOLESTEROLEMIA FAMILIES AND FOUR POLYMORPHISMS.

Emi YAMAKI, Mitsuru EMI, Tsunenori HIRAYAMA (Inst. Gerontol., Nippon Med. Sch., Kawasaki), Yachio OHTA (Tenshi Hosp), Masahiro TSUJI (Int. Med., Hokkaido Social Security Cent. Hosp., Sapporo) and Akira HATA (Hokkaido Univ. Sch. Med., Sapporo)

Familial hypercholesterolemia (FH) is a common autosomal dominant disorder that is caused by a mutation in the gene for the low density lipoprotein (LDL) receptor. We have identified mutations of the LDL receptor gene in three FH families by PCR-SSCP analysis, direct DNA sequencing, and Southern hybridization of all 18 exons. In the first heterozygous FH family, three sisters had a nonsense mutation at codon 95 in exon 3. In the second heterozygous FH family, mother and son had a missense mutation (pro-ser, C-to-T transition) at codon 608 in exon 12. The founder of the last family was a male at 22-year-old with the plasma cholesterol level of 684 mg/dl and the cutaneous xanthomas. One mutant allele having 2.5-kb insertion around exon 11 was detected by Southern hybridization. Another allele is to be examined. We also report four polymorphisms in the exons of the LDL receptor gene ; (1) AGG-AGA at codon 471, (2) GTC-GTT at codon 653, (3) CCG-CGA at 744, (4) G-A at +52 in 3'UTR region.

A-29

MUTATIONS AT 8993 OF MITOCHONDRIAL DNA IN LEIGH'S SYNDROME
Michiko MAKINO, Ikuya NONAKA, Yu-ichi GOTO
(Dept.Ultrastructural Res., Natl.Inst.Neuroscience.,
NCNP, Tokyo)

We studied point mutations at 8993 of mitochondrial DNA in 60 Japanese patients with Leigh's syndrome. We detected the mutations in 8 of 60 patients in heteroplasmic fashion. Seven of them showed a T>G mutation and one patient revealed a T>C mutation at 8993. We examined all the muscle biopsies from 8 patients histologically, histochemically and biochemically. In one patient cytochrome c oxidase activity was decreased on both histochemistry and biochemistry. One patient had a combined biochemical defect with complex I and cytochrome c oxidase (complex IV) deficiency. These results indicate that the 8993 mutations are relatively common in Leigh's syndrome and also that muscle histochemistry and mitochondrial enzyme activities revealed essentially normal.

A-30

MITOCHONDRIAL DNA VARIANTS OBSERVED IN SPORADIC ALZHEIMER DISEASE PATIENTS. Kyoko TATEISHI, Kouzin KAMINO, Aoi YOSHIIWA, Yumiko NISHIWAKI, Tetsuro MIKI, Toshio OGIHARA (Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka) and Hideki YAMAMOTO, Toshiko KOBAYASHI (Osaka Municipal Kohnsain Hospital, Osaka)

To elucidate the relationship between mitochondrial variants and Alzheimer disease, patients with senile dementia of Alzheimer's type and non-demented controls in Japanese population were screened for the variants by PCR-RFLP. Among mitochondrial variants identified in Caucasian patients with Alzheimer and/or Parkinson disease described by Wallace and his colleagues, ND2⁴⁷³² and ND2⁵⁴⁶⁰ were found, while 12S rRNA^{956-965ins}, 12S rRNA⁷²¹, 16S rRNA³¹⁹⁶, ND1³³⁹⁷, tRNA^{Gln4336} and ND5 variants were not. Novel variants were also identified; 12S rRNA⁸⁵⁶, 12S rRNA¹⁰⁰⁵ and ND2⁵⁴⁶⁵. Although our results did not support the hypothesis that the variants of mitochondrial genes encoding NADH dehydrogenase and tRNA^{Gln} are related with Alzheimer disease, we proposed that novel mitochondrial variants of 12S rRNA⁸⁵⁶ and 12S rRNA¹⁰⁰⁵ are possible risk factors for SDAT.

A-31

Tissue specificity in the processing of the mitochondrial polycistronic RNA at the tRNA^{Leu(UUR)} gene boundary. Yasutoshi KOGA and Hirohisa KATO.
(Dept. Pediat. Kurume Univ. Fukuoka)

We described the novel unprocessed RNA species (RNA19), corresponding to the genes of 16S rRNA + tRNA^{Leu(UUR)} + ND 1 genes. In this study, we have analyzed the steady state levels of unprocessed RNA intermediates at tRNA^{Leu(UUR)} gene boundary, to know the biological meanings of this novel mitochondrial transcripts. Total RNA was isolated, electrophoresed, transferred, and hybridized with 16R rRNA, or tRNA^{Leu(UUR)}, or ND 1 probes. The RNA 19 is recognized in all human tissues including muscle, heart, brain, kidney, liver, spleen and uterus, ranging 3.5%, 11.6%, 13.6%, 8.4%, 4.2%, 1.7%, and 6.5% of their total ND 1 signal, respectively. Using tRNA^{Leu(UUR)} as a probe, transcript corresponding to tRNA^{Leu(UUR)} + ND 1 is a dominant molecules in heart, muscle and liver, kidney, however, transcripts corresponding to 16S rRNA + tRNA^{Leu(UUR)} is dominant in cultured skin fibroblasts. Our data suggest that mitochondrial processing system at tRNA^{Leu(UUR)} boundary may be influenced by tissue specific manner. Since their steady state levels varied, it may contribute the oxygen capacity of the tissues, which may play an important role of the mitochondrial respiratory capacity in the tissues.

A-32

Mitochondrial DNA polymorphism in East Asian populations with special reference to the peopling of Japan. Satoshi HORAI (National Institute of Genetics), Goonnappa FUCHAROEN (Khon Kaen Univ.), Shinji HARIHARA (Univ. of Tokyo), Kyung Sook PARK (Sung-Shin Women's Univ.), Keiichi OMOTO (International Center for Japanese Studies) and I-Hung PAN (National Taiwan Univ.)

Nucleotide sequences of the D-loop region of human mitochondrial DNA from five East Asian populations including mainland Japanese, Ainu, Ryukyans, Koreans and Chinese were analyzed. Based on a comparison of 482 base pair sequences in 293 East Asians, 207 different sequence types were observed. Of these, 189 were unique to their respective populations, whereas 18 were shared between two or three populations. Among the shared types, eight were found in common between the mainland Japanese and Koreans, which is the largest number in the comparison. Phylogenetic analysis revealed that East Asian lineages were classified into at least 18 monophyletic clusters, though lineages from the five populations were completely intermingled in the phylogenetic tree. However, we assigned 14 out of the 18 clusters for their specificity based on the population from which the maximum number of individuals in each cluster was derived. Of note is the finding that 50% of the mainland Japanese had continental specificity in which Chinese or Koreans were dominant, while less than 20% of either Ryukyans or Ainu possessed continental specificity. Phylogenetic analysis of the entire human population revealed the closest genetic affinity between the mainland Japanese and Koreans. Thus, the results of this study are compatible with the hybridization model on the origin of modern Japanese. It is suggested that about 65% of the gene pool in mainland Japanese was derived from the continental gene flow after the Yayoi Age.

A-33

THE APPLICATION OF GENETIC BACKGROUND ANALYSIS USING MITOCHONDRIAL DNA AND SKEWED PREVALENCE OF COMMON MUTATIONS IN MPS VIA Zenichiro KATO, Seiji FUKUDA, Shunji TOMATSU, Atsushi YAMAGISHI, Naoto YAMADA, Kazuko SUKEGAWA, Tadao ORII, Naomi KONDO. (Department of Pediatrics, Gifu University School of Medicine, Gifu)

We analysed the genetic background of MPS IV A using mtDNA lineages in association with common mutations of the disease-causing gene. We compared the nucleotide sequence of the major non-coding region of patients' mtDNA with reported lineages. Colombian and Mexican patients lineages fall into distinct clusters observed in native-Americans with some variation. Caucasian (British, Irish and Australian) and Japanese patients had similar lineages to the reported lineages in the respective populations. We have identified a novel common mutation, G301C, in 68 % of unrelated mutant alleles of Colombia, but not in the other populations. The haplotype analysis suggested the founder effects of the common mutation. The skewed prevalence of G301C mutation can be explained as a founder effect during the Mongoloid migrations that contributed to the colonization of Colombia. The mtDNA lineage analysis can pave the way to obtain a quantitative information about genetic background of the patients. Moreover, it can be applied for the other many inherited diseases even if they don't have informative intragenic polymorphisms.

A-34

POLYMORPHISM OF THE ALDOSE REDUCTASE GENE IS ASSOCIATED WITH ALDOSE REDUCTASE PROTEIN LEVELS AND DIABETIC MICROANGIOPATHY Makiko OGATA¹, Naoko IWASAKI¹, Masao KANAMORI², Sachiyo KARIBE¹, Yoshiatsu TAKAHASHI¹, Hisako OHGAWARA¹, Yasue OMORI¹ Diabetes Center¹ and Medical Research Institute³, Tokyo Women's Medical College, Department of Hygiene, Toho University²

Glycation thought to affect the development of diabetic complications such as retinopathy, nephropathy and neuropathy. Here we have measured erythrocyte aldose reductase (AR) protein levels and genotyped AR gene in 140 NIDDM patients, without overt renal dysfunction or the history of AR inhibitors. Erythrocyte AR protein levels were measured by two-site ELISA using recombinant human AR. The microsatellite polymorphism in the 5'-flanking region of the AR gene has been typed using PCR. Average protein levels of AR in NIDDM group was 9.7 ± 3.2 (mean \pm SD) ng/mgHb, which is nearly equal to the reported data for the normal controls. Tandem C-A repeats of the AR gene were highly polymorphic, 9 alleles were observed in NIDDM group and 10 alleles in 74 normal control subjects. Patients who have allele 5 and 10, the latter one is not observed in Chinese population showed higher AR levels than others, respectively. The frequency of preproliferative or proliferative retinopathy (PDR) was 4.85 folds higher in patients whose AR levels were over 12.0 ng/mgHb compared to those whose levels were lower than 7.4 ng/mgHb, significantly. The size of allele 5 is 136 base pairs which was correspond to the size previously reported to associate with retinopathy in Chinese. We have observed that the number of patients with allele 10 were frequent among the group who have PDR. Nephropathy was also frequent among the patients who have allele 10. These results show that the AR protein levels are regulated by genetic factors and associate to retinopathy and nephropathy in NIDDM.

A-35

GENETIC STUDY ON SUSCEPTIBILITY TO PARKINSON'S DISEASE. Yuji MORIMOTO, Ikuko KONDO, Fumie TAKAKUBO, Kazuhiro SHIGEMOTO(Dept. of Hygiene, Ehime Univ., Ehime), and Mitsutoshi YAMAMOTO(Dept. of Neurol. Kagawa Pref. Cen. Hosp., Kagawa)

Parkinson's disease is a multifactorial neurodegenerative disorder. A negative association between PD and cigarette smoking has been reported epidemiological studies of risk factors for PD. In addition, nicotine stimulates dopamine release. Cytochrome P450IA1 plays an important role in the activation of mutagens such as benzo[a] pyrene and aromatic hydrocarbons found in cigarette smoke. DNA mutations in 3' flanking region(MspI polymorphism) and exon 7 of CYPIA1 gene(genotypes of Ile and Val) are associated with inducible phenotype of CYPIA1 enzyme. Then, We studied on the association between CYPIA1 genotypes and susceptibility to PD. The frequency of MspI polymorphic allele, a variant of CYPIA1 (m2), was significantly higher in patients with PD(0.444) than in controls(0.349). The risk of PD in homozygotes for m2 was 2.34 fold greater than homozygotes for the wild type, m1. The relative risk for PD in homozygotes for CYPIA1Val was 6.54-fold higher than in homozygotes for the wild type(CYPIA1Ile) (p<0.001). These results strongly suggest that the CYPIA1 might be one of the susceptibility genes for PD.

A-36

NO ASSOCIATION BETWEEN A POLYMORPHISM OF THE ENDOTHELIAL CONSTITUTIVE NITRIC OXIDE SYNTHASE GENE AND ISCHEMIC STROKE IN A JAPANESE POPULATION. Yoshitsugu YAHASHI (Div. Genet., ICMR, Kobe Univ. Med., Hyogo), Kazuomi KARIO (Dept. Cardiol., Jichi Med. Sch., Tochigi), Masafumi MATSUO (Div. Genet., ICMR, Kobe Univ. Med., Hyogo)

Endothelial-derived nitric oxide (NO) formed by endothelial constitutive nitric oxide synthase (ecNOS) mediates vasodilation and antithrombotic action. A close association between a polymorphism of ecNOS gene (27-bp repeats in intron 4) and onset of coronary artery disease in Caucasian was reported and a close association between onset of angiopathy and this polymorphism was suggested. We analyzed this polymorphism in 127 ischemic stroke patients (18 atherothrombotic, 58 lacunar, 51 silent lacunar) and 91 healthy subjects. We identified 2 alleles (denoted as "allele a" for 4-repeats and "allele b" for 5-repeats), and grouped the subjects into 3 genotypes (a/a, b/a, b/b). There was no significant difference in the genotype distribution and allele frequencies between the stroke and control groups. There was also no significant difference in the genotype distribution and allele frequencies between the stroke types. From these findings, we could not find the evidence that this ecNOS gene polymorphism may be a genetic factor for the onset of cerebrovascular disease.

A-37

THE POLYMORPHISM OF ARYLAMINE N-ACETYLTRANSFERASE AND O⁶-METHYLGUANINE-DNA METHYLTRANSFERASE IN ALZHEIMER DISEASE

Masanobu OGAWA, Ryo INOUE, Masako ABE, Toshihiro YOSHIKAWA* and Tomokazu SUZUKI (Dept. Clin. Genet., Med. Inst. of Bioregulation, Kyushu Univ., Beppu and *Dept. Neurol., Univ. Tsukuba, Tsukuba)

N-acetylation polymorphism is a representative of pharmacogenetic traits determined by polymorphic arylamine N-acetyltransferase (NAT2) activity. O⁶-methylguanine-DNA methyltransferase (MGMT), which repairs the DNA damages caused by alkylating agents, has also polymorphism in the MGMT gene. There are still some genetic loci that need to be identified in Alzheimer disease (AD). We have investigated the possible association between the polymorphisms of the two xenobiotic enzymes and AD. Using the genomic DNA extracted from leukocytes in 81 patients with AD and 645 normal subjects, the acetylator genotype was determined by a PCR-RFLP method and the polymorphism in the MGMT gene was detected by a PCR-SSCP method, as we described before. The distribution of presumed acetylator phenotype in patients with AD was not significantly different from that in normal subjects. There was also no significant difference in the prevalence of mutated MGMT allele between AD patients and normal subjects. In conclusion, neither polymorphism of NAT2 nor MGMT might be ecogenetically involved in AD.

A-38

A GENOME-WIDE SCREEN FOR NIDDM-SUSCEPTIBILITY GENES IN JAPANESE. Naoko IWASAKI, Makiko OGATA, Yumiko SAKISAKA, Yasue OMORI (Diabetes Center, Tokyo Womens Medical College) Hisako OHGAWARA (Medical Research Institute, Tokyo Womens Medical College) Nancy J. COX, Graeme I. BELL (Howard Hughes Medical Institute, The University of Chicago)

Genetic factors contribute to the development of NIDDM. We have begun a genome-wide screen to localize the genes that contribute to the development of this form of diabetes mellitus in Japanese. The study population consisted of 264 affected sib pairs (142 sibships with 2 affected, 22 with 3 affected, 6 with 4 affected and 2 with 5). A diagnosis of NIDDM was made using the criteria of WHO. The age at diagnosis was 44.3±11.7 years, duration of diabetes was 13.5±9.2 years, BMI at the time of study was 23.2±3.3, and current treatment of diabetes was 31% diet, 38% oral hypoglycemic agent, 31% insulin. Subjects have been genotyped with polymerase chain reaction based microsatellite markers. Evidence for linkage has been evaluated using two different two-point linkage statistics, a maximum likelihood of odds score (MLOD) computed using the estimated identical-by-descent (IBD) probabilities and a chi-square statistic comparing the observed and expected distribution of alleles identical-by-state (IBS). A multipoint analysis has been used to maximize the information from each chromosome and provide a bias for exclusion. Of 150 markers typed to date, markers D2S2366, D2S1391, D15S112, D21S1270, D21S1440, D21S266 and D22S420 showed nominal evidence for linkage (P<0.05). Suggestive and significant evidence for linkage require P< 7×10⁻⁴ and P< 2×10⁻⁵, respectively). Candidate genes have been tested have not shown any evidence for linkage. The genome-wide screen is continuing in order to localize all the NIDDM susceptibility loci segregating in this population.

A-39

An Association between the Angiotensin Converting Enzyme Gene Polymorphism and Parameters of Circulation System. Yingning ZHANG¹, Zentarō YAMAGATA², Sumio IJIMA¹, and Akio ASAKA¹

(¹Dept. of Health Sciences, Yamanashi Medical University, and ²Health Care Center, Yamanshi University)

We studied an association between angiotensin converting enzyme (ACE) gene polymorphism and parameters of circulation system on 384 healthy unrelated Japanese aged from 21 to 89 years (mean=58.5±14.2). Insertion (I) and deletion (D) allele in ACE gene was determined by PCR method and parameters of circulation system such as total cholesterol, HDL-cholesterol, and blood pressure were determined in the group. The gene frequency was 0.624 for allele I and 0.376 for allele D. This population was in Hardy-Weinberg equilibrium. Values of total cholesterol were 196.1mg/dl, 194.9 mg/dl, and 191.9mg/dl in the subjects who have genotype II, ID, and DD, respectively. HDL-cholesterol was 52.7mg/dl, 52.4mg/dl, and 54.2mg/dl in genotype II, ID, and DD subjects, respectively. Furthermore, we demonstrated that subjects who carried allele I had more platelet numbers than allele D gene. But this variety is not significant difference. Finally, our result showed that there was no association between ACE genotype and blood pressure.

A-40

POLYMORPHISM OF THE ANGIOTENSINOGEN AND ANGIOTENSIN II TYPE 1 RECEPTER GENES IN JAPANESE PATIENTS WITH PREGNANCY INDUCED HYPERTENSION. Naoki YAMADA, Satoshi SOHDA, Hiromi HAMADA, Takeshi KUBO (Dept.Obstet. Gynecol.,Inst.Clinical

Med.,Univ.Tsukuba),

Tadao ARINAMI, Trista Y OU, Kimiko KOBAYASHI, Hideo HAMAGUCHI (Dept.Med.Genet.,Inst.Basic Med. Sci., Univ. Tsukuba)

The purpose of the present study was to assess the association of pregnancy induced hypertension (PIH) with the M235T polymorphism of the angiotensinogen gene (AGT), T174M polymorphism of the AGT, and A1166C polymorphism of the angiotensin II type 1 receptor gene (AT1). The subjects were 67 PIH patients, 56 normotensive pregnant subjects, and 380 control subjects. No significant differences in the frequency of C1166 allele of the AT1 gene (0.04 vs 0.04, p=0.79) and T235 allele of the AGT gene (0.77 vs 0.85, p=0.11) were observed between PIH and pregnant controls. The M174 variant allele has significantly increased in the patients group compared with controls (0.16 vs 0.07, p=0.006). The M174 variants also tended to increase in the patients compared with the normotensive pregnant controls. This results suggest the contribution of the M174 variant of the angiotensinogen gene to the occurrence of PIH.

A-41

METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM AND PREECLAMPSIA. Satoshi SOHDA, Hiromi HAMADA, Takeshi KUBO (Dept. Obstet. Gynecol., Univ. of Tsukuba, Ibaraki), Trista OU, Kimiko YAMAKAWA-KOBAYASHI, Tadao ARINAMI, Hideo HAMAGUCHI (Dept. Med. Genet., Univ. of Tsukuba, Ibaraki)

A common missense mutation in the MTHFR gene, a C-to-T substitution at nucleotide 677, is responsible for reduced MTHFR activities and associated with modestly increased plasma homocysteine concentrations. Since underlying maternal vascular disease increases the risk of preeclampsia, we had the working hypothesis that preeclampsia patients would have an increased T677 allele frequency compared with controls. The MTHFR genotypes were determined in 67 preeclampsia patients, 98 normal pregnant women and 260 healthy adults by the PCR/RFLP methods. The T677 allele and the genotype homozygous for the T677 allele were significantly increased in the preeclamptic group compared with the total controls ($p < 0.02$ and $p < 0.004$, respectively,).

The data indicate that the T677 variant of the MTHFR gene is one of the genetic risk factors for preeclampsia.

A-42

SYNERGISTIC EFFECTS OF METHYLENETETRAHYDROFOLATE REDUCTASE AND APOLIPOPROTEIN E GENE POLYMORPHISMS ON RISK OF CORONARY HEART DISEASE. Trista Y. OU, Naoki YAMADA, Kimiko KOBAYASHI, Tadao ARINAMI, Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med. Sci. Univ. Tsukuba, Tsukuba)

It has been reported that increased plasma homocysteine levels are associated with coronary heart disease(CHD). A missense polymorphism, C677T(Ala→Val), of the methylenetetrahydrofolate reductase(MTHFR) gene is responsible for thermolabile MTHFR variant which causes increased levels of plasma homocysteine. To examine whether the MTHFR polymorphism is associated with CHD in Japanese populations. We analyzed it in 187 CHD patients and 362 control subjects. Our data showed the TT genotype was significantly increased in the CHD patients compared with the control subjects (odds ratio=2.3 $p < 0.001$). The allele frequency of apoE4 which is associated with increased levels of LDL cholesterol tended to increase in the CHD patients than in the controls (odds ratio=1.5 $p = 0.05$).

In addition, the effect of the MTHFR TT genotype on risk of CHD increased in subjects with the apoE4 allele compared with those without the allele. Thus, synergistic effects of the MTHFR TT genotype and apoE4 allele on risk of CHD was suggested (odds ratio=6.2 $p = 0.001$).

A-43

GENE POLYMORPHISMS OF RENIN ANGIOTENSIN SYSTEM IN HEMODIALYSIS PATIENTS WITH GLOMERULAR DISEASE. Makiko FUNAMIZU¹, Akira HATA¹, Takamichi NAKAMURA², Munemasa TAJIRI³, Haruo YAMASHITA⁴, Kensuke TAKADA⁵, Yoshihei HIRASAWA⁶, Koichi SHIDO¹, and Kiyotaro KONDO¹ (¹Dept. Public Health, Hokkaido Univ. School of Med., Sapporo, ²Div. Blood Transfusion, Yamanashi Med. University, ³Kurokami Clinic, Kumamoto, ⁴Yamanashi Chuo Hosp., ⁵Fujiyoshida City Hosp., ⁶Shinrakuen Hosp., Niigata)

Renin angiotensin system (RAS) has been considered to play an important role in renal disease progression. To investigate the influence of the components of RAS in the progression, we genotyped polymorphisms of angiotensinogen (AGT: M235T), angiotensin converting enzyme (ACE: I/D), and type I angiotensin II receptor (AIIR: A1166C) genes in 212 hemodialysis patients (age 46.7 ± 13.0) and 189 sex- and age-matched healthy controls (age 47.8 ± 12.5). The native kidney disease that led to end stage renal failure was of glomerular origin, including 158 patients with chronic glomerulonephritic syndrome (CGNS) and 54 patients with diabetic nephropathy (DN). The frequencies of DD genotype of ACE gene and TT genotype of AGT gene in CGNS, DN, and controls were 0.09, 0.07, 0.08 and 0.63, 0.58, 0.66, respectively. Those of AC+CC genotypes of AIIR were 0.16, 0.17, and 0.13. None of the differences was significant, which may indicate that these three components of RAS are not a major risk factor for renal disease progression.

A-44

RELATION OF VITAMIN D AND VITAMIN D RECEPTOR GENE POLYMORPHISMS ON POSTMENOPAUSAL BONE LOSS. Makoto OWADA, Kumiko SUZUKI, Satoshi ONOGI, Hidekazu YAMADA, Susumu Tsukikawa, Kazuhiko HOSHI and Akira SATO (Dept. Obstet. Gynecol., Fukushima Medical College, Fukushima)

In order to assess the effect of vitamin D receptor (VDR) gene polymorphisms on vitamin D3 therapy for postmenopausal bone loss. 34 Japanese postmenopausal women, administered vitamin D3 (Alfarol $1.0 \mu\text{g/day}$) and Ca (2.0g/day) for 18 months, were analyzed by RFLP method. Bone mineral density (BMD) at the lumbar spine (L2-4) and Os-calcis were measured every 6 months by dual energy X-ray absorptiometry (DXA) and single energy X-ray absorptiometry (SXA). VDR-gene allelic polymorphisms were assessed by Bsm 1 endonuclease restriction after specific PCR amplification. Genotypic polymorphism was defined as BB, bb and Bb. The genotypes were BB in 1 (3.1%), Bb in 13 (40.6%), and bb in 18 (56.3%). The women with these two major VDR genotype groups (Bb and bb) were similar in their backgrounds (terms of age, menopausal age, body mass index, and BMD in premedication). However, VDR genotype was associated with the percent of change of BMD after treatment. In Group-Bb, the mean percent increase in L2-4 BMD were 3.2%, 4.9% and 4.1% at each 6, 12 and 18 months. In contrast, in Group-bb were 0.8%, 1.8% and 1.2% at the same points. Analysis of VDR alleles may prove useful in selection of vitamin D therapy for osteopenia before treatment.

A-45

VITAMIN-D-RECEPTOR-GENE POLYMORPHISMS ARE ASSOCIATED WITH OSTEOPOROSIS IN JAPANESE WOMEN. Hisako YANAGI¹, Hitoshi AMAGAI², Shigeo TOMURA¹, Kimika KAWANAMI¹, Miwa HOSOKAWA¹, Mari TANAKA¹, Kei KOBAYASHI¹, Koichiro HAYASHI², Hideo HAMAGUCHI³ and Shigeru TSUCHIYA¹ (¹Institute of Community Medicine, ²Institute of Clinical Medicine, ³Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba)

Polymorphisms of the vitamin-D-receptor (VDR) gene have been shown to be associated with bone mineral density (BMD). We analyzed the VDR alleles in 68 Japanese female patients with osteoporosis and 66 age-matched volunteer women. VDR-gene allelic polymorphisms were assessed by *BsmI* endonuclease restriction after specific PCR amplification. Sixteen out of 68 patients had the BB (absence of restriction site on both alleles) genotype, while only two out of 66 controls had the BB genotype. The difference was significant ($p < 0.001$). Odds ratios for osteoporosis of bb, Bb and BB were 1.0, 3.0 and 12.0, respectively. These data suggest that BB homozygosity of the VDR gene is a strong genetic risk factor for osteoporosis in Japanese women.

A-46

AN ASSOCIATION BETWEEN ESTROGEN RECEPTOR GENE POLYMORPHISMS AND BONE MINERAL DENSITY. Zentaro YAMAGATA (Health Care Center, Yamanashi University), Yingning ZHANG, Toshihiro MIYAMURA, Sumio IIJIMA, Akio ASAKA (Dept. of Health Sciences, Yamanashi Medical University)

To examine a possible influence of estrogen receptor (ESR) gene polymorphisms on bone mineral density (BMD) we analyzed two gene polymorphisms within ESR gene in 107 healthy Japanese women. Two polymorphisms were that of *PvuII* and *XbaI* restriction enzymes by PCR in intron 1. BMD were measured dual energy X-ray absorptiometry. They were 15.0% PP homozygotes, 52.3% Pp heterozygotes and 32.7% pp homozygotes for the *PvuII* polymorphism, and the frequency of genotype for *XbaI* polymorphism were 2.8% for XX, 39.3% for Xx and 57.9% for xx. BMD adjusted by age, menopause status, weight and VDR genotypes increased in the order pp, Pp and PP ($F=3.59$, $p=0.032$), and increased in the order xx, Xx and XX ($F=13.12$, $p=0.0001$). BMD adjusted by age, menopause status, weight and VDR genotypes in haplotype XXPP was significantly higher than one in the other genotypes. This study indicated that ESR gene polymorphisms had an influence on BMD in Japanese women.

A-47

AN ASSOCIATION STUDY ON ESTROGEN RECEPTOR GENE POLYMORPHISM WITH BREAST CANCER Masao KANAMORI*, Zentaro YAMAGATA**, Takashi FUKUTOMI***, Akio ASAKA****, Yingning ZHANG****, Minatu KOBAYASHI*, and Minoru SUGITA*, (*Dept. Environ. Health, Toho Univ. School of Med., Tokyo, **Health Care Center, Yamanashi University, Yamanashi, ***Dept. Surgery, National Cancer Center Hospital, Tokyo, ****Dept. Health Sciences, Yamanashi Medical University, Yamanashi)

The common hereditary forms of breast cancer(BC) have been largely attributed to the mutations in BRCA1 or BRCA2. We have studied on an influence of estrogen receptor(ESR) gene polymorphisms on breast cancer. 43 cases (who visited National Cancer Center Hospital on September, 1996, it is a spot value, no selection bias) and 173 healthy controls are surveyed. PvuII and XbaI restriction enzymes in intron 1 are examined by PCR. The results were as follows: 1) a significant association between PvuII and XbaI polymorphisms in healthy controls. 2) not significant differences between frequencies of PvuII and XbaI genotypes in BC patients and in controls. 3) Assuming PvuII and XbaI polymorphisms as independent variables, the relative risk (odds ratio) of pair of homozygotes with haplotypes p and x to other pairs have been elevated.

A-48

ASSOCIATION STUDY ON OSTEOPOROSIS WITH THE MICROSATELLITE MARKER POLYMORPHISMS FLANKING THE CANDIDATE GENES. Kazuhiro TSUKAMOTO, Ken HARUTA, Mitsuru EMI (Dept. Mol. Biol., Inst. Gerontol., Nippon Med. Sch., Kawasaki), Takayuki HOSOI (Dept. Geront., Univ. Tokyo, Tokyo) and Hajime ORIMO (Minist. Finance, Tokyo Hosp., Tokyo)

The etiology of osteoporosis involves a complex interplay of exogenous and endogenous factors, including genetic factors. The identification of candidate genes has helped to refine the characterization of osteoporosis. We carried out an association study between bone mineral density (BMD) as well as biochemical parameters for bone metabolism and the microsatellite (CA repeat) marker polymorphisms which we newly isolated flanking candidate genes, calcitonin, parathyroid hormone (PTH), PTH receptor, interleukin 6 (IL6), IL6 receptor, interleukin 1 alpha, beta, and estrogen receptor (ER). We detected P1-derived artificial chromosome (PAC) clones including the sequence of the candidate gene by PCR-based 3D screening of human PAC DNA library. These PAC clones were subcloned into the plasmid vectors after endonuclease digestion and the DNA fragments including the CA repeat sequence were detected by (GT)₂₀ oligonucleotide hybridization. After sequencing, the primer pair flanking the CA repeat was newly designed for PCR. Correlation was sought between ER genotype C (the number of CA repeat was 16) and BMD as well as biochemical parameter.

A-49

DETECTION OF t(11;14)(q13;q32.33) IN INTERPHASE NUCLEI OF MANTLE CELL LYMPHOMA BY IN SITU HYBRIDIZATION. Teruyuki TAKASHIMA¹, Yutaka UEDA¹, Shigeo HORIIKE¹, Kazuhiro NISHIDA¹, Shinichi MISAWA¹, Tatsuo ABE², Masao SETO³, and Masafumi TANIWAKI¹ (¹Dept. Internal Medicine and ²Dept. Hygiene, Kyoto Prefectural University of Medicine, Kyoto, and ³Dept. Chemotherapy, Aichi Cancer Center Research Institute, Nagoya)

To assess the incidence and pathogenetic implication of t(11;14)(q13;q32.33) in mantle cell lymphoma (MCL), we performed fluorescence in situ hybridization (FISH) with PRAD1/CCND1 and IgH gene probes. Twenty-eight patients were studied; 6 with leukemic phase of MCL, 18 patients with chronic lymphocytic leukemia (CLL), 2 with CLL of mixed cell types (CLL/PL), 2 with prolymphocytic leukemia (PLL). Double-color FISH was performed with 2 cosmid clones, CPP4 and CPP29 containing PRAD1 gene, in combination with C γ or V_H gene probes. Five patients with MCL, 1 with CLL/PL, and 1 with PLL showed t(11;14). In a patient with PLL, the breakpoint was in 5' flanking region of PRAD1 gene. Interphase FISH facilitates to correlate molecular characteristics of t(11;14) with clinical features of B-cell leukemia/lymphomas.

A-50

ANALYSIS OF CHROMOSOMAL ABERRATIONS IN THYROID TUMORS BY FISH. Takashi SHINOMIYA^{1,2}, Satosi TAKAMI⁵, Toshiki MORI¹, Kousei DATE¹, Kazuma KOIDE¹, Shinsuke ISHINO¹, Youhei HOSOKAWA⁴, Norio YASUDA³, Yasushi MURAKAMI², Johji INAZAWA¹, Tatsuo ABE¹ (Dept. Hygiene¹ and Otorhinolaryngol.², Kyoto, Pref. Univ. of Med., Dept. Otorhinolaryngol.³, and Pathol.⁴, Kyoto 1st Red Cross Hosp., Ohtsuka Assay Inst.⁵.)

Fluorescence in situ hybridization (FISH) with probes specific for (peri)centromeric regions of chromosomes 1, 2, 7, 8, 10, 11, 17, 18 was performed to detect numerical chromosomal aberrations in 24 thyroid tumors and tumor-like lesions. In addition, double target FISH with probes specific for 1p36 and 1q12 was used for the detection of loss on 1p. The nuclear specimens were obtained by fine-needle aspiration of the fresh surgical tissue. Numerical aberrations were found in 3 of 14 papillary carcinomas (PC), 2 of 3 follicular carcinomas (FC), 3 of 4 benign follicular lesions. We added LOH study on 2p21 (D2S123), 3p14.3-21.1 (D3S1067), 17p13.1 (TP53) in a case with polysomy of chromosome 1, 7, 10, diagnosed as adenomatous goiter at first. LOH was found on 3p but not 2p and 17p. This case was reexamined pathologically, and finally diagnosed FC, minimally invasive. FISH analysis of fine-needle aspiration may provide useful information for the preoperative diagnosis of thyroid tumors.

A-51

FREQUENT MULTIPLICATIONS OF THE LONG ARM OF CHROMOSOME 1 IN HUMAN BREAST CANCERS

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We performed FISH to investigate the copy number of chromosomes 1, 8, 11, 17 as well as deletions of chromosome 1p in human breast cancers. These data were analyzed with respect to the histological grade in each tumor. Gains of copy number of chromosome 1 were the most frequent events among three different grades, whereas numerical aberrations of chromosomes 8, 11, 17 were closely related to the up-grading. Furthermore PCR-LOH analyses throughout chromosome 1 revealed that allelic imbalance was detected on the long arm of chromosome 1, but not on the short arm in all low-grade breast cancers examined. These results suggest that the gain of 1q on one allele may be an even earlier event among the several types of specific chromosomal aberrations in breast cancers previously reported.

A-52

CHROMOSOME ANALYSIS OF MALIGNANT ASTROCYTOMA CELL LINE (U251-MG) BY COMPARATIVE GENOMIC HYBRIDIZATION. Toshiki MORI^{1,2}, Chohei SAKAKURA¹, Youji ARIYAMA¹, Takashi SHINOMIYA¹, Kazuma KOIDE¹, Kousei DATE¹, Satoshi UEDA², Johji INAZAWA¹, Tatsuo ABE¹ (¹Depts. of Hygiene and ²Neurosurgery, Kyoto Pref. Univ. of Med.)

Chromosomal aberrations of two neuroectodermal tumor cell lines were analyzed by comparative genomic hybridization (CGH). The materials were a malignant astrocytoma cell line (U251-MG) and a neuroblastoma cell line (RTR). Sample DNAs extracted from the cell lines and normal DNA from normal lymphocytes were labeled with two different fluorochromes of Spectrum green and Spectrum red by nick-translation, respectively, and then the both were cohybridized to metaphase chromosomes. Imaging and analyses of chromosomal losses, gains and amplifications for them were carried out by using MacProbe and MacKaryotype (PSI). In U251-MG, copy number gains were observed on chromosomes 1p13.1-13.2, 1q21.1-21.2, 3q27-29, 4q-12, 6, 7, 17q, 19q, X and copy number losses on 11q, 18q, 20p, 21 and Y. RTR showed copy number gains on 2p24, 5p, 17q, 20q, 21q, 22q and copy number losses on 1p, 18 and Y. Amplifications were clearly detected, however, deletions were detected only in the metaphase hybridized properly. CGH was proved to be useful for chromosomal analysis.

A-53

ANALYSIS OF CHROMOSOMAL ABERRATION IN HUMAN GASTRIC CANCERS Chouhei SAKAKURA^{1,2}, Toshiki MORI², Yohji ARIYAMA², Yoshiteru ARAKAWA², Kazuma KOIDE^{1,2}, Takashi SHINOMIYA², Yukio DATE², Akeo HAGIWARA¹, Toshiharu YAMAGUCHI¹, Toshio TAKAHASHI¹, Tatsuo ABE² and Johje INAZAWA² (Kyoto Prefectural University of Medicine, ¹Department of Hygiene, ²First Department of Surgery)

In order to screen for DNA sequence copy number changes in gastric cancer, CGH analysis was performed in 42 gastric cancers. In the present study CGH analysis indicated DNA sequence copy number changes in 95% of primary gastric cancers. Losses were often involved in 1p, 5q, 17p, 18. Gains were often involved in 1q, 7p, 11p, and X. Novel gene amplification was identified on 8p23.1 in about 10% of advanced gastric cancers (Borrman 3 and 4), suggesting that this region may contain dominant gene related to the progression of gastric cancer. CGH is powerful tool for direct orsualization of chromosomal loss and gain among all chromosomes.

A-54

EVALUATION ON ABILITY OF ABNORMALITY DETECTION BY CGH METHOD IN TUMOR CHROMOSOMES AND ITS METHODOLOGICAL IMPROVEMENT
Kiyomi YAMADA (Dept Genetics, Res Institute, Nat Inter Med Cent Japan), Fumihiro ASANO, Hiroshi YAMAZAKI (Koutoubiken Central Laboratory, Tsukuba), Eiji ISHIZUKA, and Youichi KISHI (Dept Urology, Hospital, Nat Inter Med Cent Japan, Tokyo)

Comparative genomic hybridization (CGH) is said to be useful for screening losses and gains of chromosomal regions along all chromosomes of tumor cells. We have investigated on accuracy and reproducibility of this method using two tumor cell-lines mostly; lung cancer cells with a sharp mode of 67 chromosomes and brain tumor cells with 54 chromosomes. In both cell-lines, all chromosome abnormalities were completely identified by G-banding and FISH painting and none of cells with normal karyotype was present. Each of 10 ul of probe solution containing either 200 ng of digoxigenin-labeled normal DNA or 200 ng of biotin-labeled tumor DNA in addition to 10 ug of salmon sperm DNA and 20 ug of cot-1 DNA, was mixed in volume ratio of 1:1 or 1:1.5. CGH analysis was done using CytoVision and CGH analysis software (AI Inc.). Results: 1) Detection of gene amplification in fluorescence intensity ratio profiles was most powerfully sensitive. 2) Sensitivity to detect trisomic chromosomal regions was relatively low and insufficient. 3) By the increase of tumor DNA amount according to the cellular DNA content, detection for trisomic chromosomal regions became remarkably effective. 4) However, in this experiment supression hybridization must be enhanced especially for telomeric chromosomal regions by adding more 20-40 ug of cot-1 DNA.

A-55

Interstitial Deletion On the Long Arm Chromosome 4, del(4)(q21.22-23).
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Medical School, Tochigi)

We report the first case of an interstitial deletion of chromosome 4, del(4)(q21.22-3). The patient was a boy presenting clinical features associated with 4q- syndrome, such as low birth weight at term, micrognathia, cardiac malformations, delayed psychomotor development, clinodactyly of the 5th finger, hypoplasia of external genitalia, hypospadias, and a liver tumor. The association of liver tumor with 4q- syndrome has not been reported previously. Although pathological studies of the tumor were not performed, hepatoblastoma was most likely because of the elevated serum level of α -fetoprotein, rapid growth, and findings of Ultrasonography and CT scans. Recent molecular studies on primary hepatocellular carcinoma (PHC) have suggested the presence of tumor suppressor gene on chromosome 4, and the LOH in hepatocellular carcinoma has been observed at 4q13-26. It has been of interests that this region overlaps with the locus of human smg GDS gene, 4q21-25, that has possible relation with the molecular events of malignant transformation. The occurrence of liver tumor in the patient with interstitial deletion at 4q21.22-23 suggested that this deletion locus included the locus of tumor suppressor gene for PHC on 4q.

A-56

ORDERING OF FIVE GENES IDENTIFIED WITHIN AN AMPLIFIED DNA UNIT (17q12) IN BREAST AND STOMACH CANCER CELLS BY MEANS OF FIBER-FISH ANALYSIS. Aiko HAYASHI^{1,2}, Tomoki ISHIZUKA³, Hiroki SASAKI³, Fumiko SAITO-OHARA¹, Sei-ichi KOHNO², Masaaki TERADA³, and Tatsuro IKEUCHI¹ (¹Div. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., ²Dept. Biol., Fac. Sci., Toho Univ., ³Genet. Div., Natl. Cancer Center Res. Inst.)

FISH signals on extended chromatin fibers (ECF) released from interphase nuclei are shown to be linear, and the resolution attainable should be comparable to analysis of a straight DNA molecule itself: 1 μ m in size of the linear FISH signal under the microscope corresponds to a DNA length of 3-10 kb. We have applied this fiber-FISH method to the structural analysis of amplified DNA regions including c-ERBB2 at the chromosome 17q12 region in breast and stomach cancer cells. A total of 12 λ EMBL3 clones (20-30 kb) were isolated from the amplified DNA unit (~450 kb), each containing sequences of 5 different genes that were identified within this amplified chromosome region. ECF preparations were made by the NaOH treatment method (Fidlerova et al., 1994) with slight modifications. After repeating of 2- or 3-colored FISH experiments with various combinations of the probes, the 12 clones could be set in array where 3 regions were found to be duplicated each other and 2 clones be localized contiguously. As a result, the order of the genes within the amplified unit was determined as follows: A39 - CAB1 - C51 - c-ERBB2 - GRB7. The detailed physical map would enable to further determine the commonly amplified DNA regions in different cancer specimens.

A-57

ALTERED EXPRESSION OF p21 (WAF1/CIP1) DURING CELL DIFFERENTIATION. Tomoko HASHIMOTO¹⁾, Hidenao YAMAMOTO²⁾, Koubou SASAKI³⁾, Shu TAMURA⁴⁾, Jiro FUJIMOTO²⁾, Eizo OKAMOTO²⁾, Hisao TACHIBANA³⁾, Minoru SUGITA³⁾, Eizo KAKISHITA⁴⁾, and Jun-ichi FURUYAMA¹⁾. (1) Dept.Genetics, 2) 1st Dept.Surg, 3) 5th, and 4) 2nd Dept.Int.Med., Hyogo Coll. Med., Nishinomiya)

p21 is one of the factors that inhibit cell cycle. The expression of p21 is induced during cell differentiation and also up-regulated by wild type p53, but not by mutant p53. We examined the expression levels of p21 during differentiation of human hepatoma cell lines, in which mutation of p53 has been frequently found. In hepatocellular carcinoma (HCC) cell lines in a log phase, lower levels of p21 mRNA were found in cells with mutant p53 than those with wild-type p53. In hepatoblastoma (HB) cell lines with wild-type p53, p21 expression was higher than all HCC cell lines. After induction of differentiation with sodium butyrate, marked induction of p21 mRNA was observed in all HCC cell lines. In HB cell lines, however, no change in the p21 level was found. We then examined mouse P19 embryonal carcinoma cells. The level of p21 was high in a log phase and slightly decreased after neuronal differentiation with retinoic acid. These results suggest that (1) the basal levels of expression of p21 is high in immature cells and low in mature cells, (2) induction of p21 during differentiation may be independent of types of p53, and vary with the developmental stage of cells.

A-58

CHROMOSOMAL SEGMENT TRANSLOCATION IN LEUKEMIAS AND LYMPHOMAS WITH COMPLEX KARYOTYPE. Kimio TANAKA, Mariko EGUCHI, Mansur ARIF and Nanao KAMADA (Dept.Cancer Cytogenet., Res.Inst. for Radiat. Biol. & Med., Hiroshima Univ., Hiroshima)

One hundred and fifty leukemia and lymphoma patients were studied in detail by FISH to identify the associated oncogene with malignant transformation. Specific chromosome regions were translocated onto structurally abnormal chromosomes, resulting in partial tri-, tetra- or penta-somy of these regions. We called this type of translocation "segmental jumping translocation (SJT)". SJT were found in several chromosomal regions of 8q24, 9q34, 11q13, 11q32, 21q22 and 22q11. SJT at 9q34, which involved ABL oncogene was found in three of eight secondary leukemia patients, who were treated with anticancer drugs and radiation. Non-Hodgkin's lymphoma patients and acute myeloid leukemia (AML) patients had 3-12 copies of SJT at 11q13 and 11q23, respectively. Those at 14q32 and 21q22 were also predominantly detected in acute type of adult T-cell leukemia (8 of 27 patients) and in AML (3 of 12 patients), respectively. Further FISH analysis using many cosmid and YAC probes revealed that size of SJT regions were variable among the patients. Overlapping region within the SJT could involve commonly associating oncogene(s) with the transformation to advanced stage in leukemias and lymphomas. The SJT suggests an evidence of new mechanism for gene amplification and formation of unidentified marker chromosomes in advanced stage.

A-59

CYTOGENETICAL AND MOLECULAR BIOLOGICAL DIFFERENCE IN CHILD AND ADULT HEMATOLOGICAL MALIGNANCIES WITH 11q23 CHROMOSOMAL ABNORMALITY. Mariko EGUCHI, Kimio TANAKA, Mansyur ARIE, Nanao KAMADA (Dept. Cancer Cytogenet., Res. Inst. for Radiat. Biol. and Med., Hiroshima Univ., Hiroshima) and Masao SETO (Lab. Chemotherapy, Aichi Cancer Res. Ins., Nagoya)

Reciprocal translocations involving chromosome 11, band q23 are frequently observed in human acute leukemia and lymphoma with more than 20 different cytogenetic loci as the partner. These leukemia are clinically distinct in that they are common in infants under one year of age, may be of either myeloid or lymphoid lineage or sometimes biphenotypic, and have a poor prognosis. Several groups have recently characterized a gene called MLL or ALL-1 that is structurally interrupted by 11q23 translocations. The cDNA probe from this region detect MLL rearrangements on conventional Southern blots.

We analyzed molecular and cytogenetical differences between child and adult 11q23 abnormal cases in 30 hematological patients. All the 13 child patients under age of 15 had a common clinical, molecular biological and cytogenetical characteristics including MLL gene rearrangements. On the other hand 17 adult leukemia and lymphoma patients were distinct from child patients in that they had more complex karyotype and less involvement of MLL gene.

FISH method was applied to identify a novel breakpoint cluster region in the 11q23 area in these MLL rearrangement negative adult hematological patients. Thirteen of the 16 patients analyzed had their breakpoint between probe 433 and 526 which is located on 11q23.1, suggesting some genetic locus in this area may be involved in 11q23 abnormal hematological disorders.

A-60

MICROSATELLITE INSTABILITY IN MYELODYSPLASTIC SYNDROME. Shinichi MISAWA, Shigeo HORIIKE, Hiroto KANEKO, Shouhei YOKOTA, Masafumi TANIWAKI, and Kei KASHIMA (Third Dept. Med., Kyoto Prefectural University of Medicine, Kyoto)

We examined microsatellite instability (MSI) at 10 loci of dinucleotide repeats using the polymerase chain reaction (PCR) in patients with myelodysplastic syndrome (MDS). Bone marrow samples were obtained from 45 patients repeatedly during the disease course and fibroblast DNA was also collected from 19 patients as a normal control. Three patients showed an alteration of MSI at 3 or more loci when the allele length was compared between the fibroblast DNA and the initial marrow DNA. On the other hand, none of the 45 patients showed an alteration between the initial marrow samples and the latest ones. The 3 patients with MSI did not show any of a disease progression, complex karyotype, karyotypic evolution, or mutation of N-RAS or TP53 gene. Moreover, a frameshift mutation of the transforming growth factor beta type II receptor gene at 10 repeating adenines, which may be critical target of MSI, was not found in any of the 45 patients. These findings suggest that MSI is an early but infrequent genetic event and is independent of other critical genetic aberrations in the development of MDS.

A-61

CHROMOSOME ABERRATION IN PATIENTS WITH MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN1). Miyuki KATAI, Akihiro SAKURAI, Yasunori ITAKURA, Kiyoshi HASHIZUME (Dept. Geriat., Endocrinol., Metab., Shinshu Univ. Sch. Med., Matsumoto) and Yoshimitsu FUKUSHIMA (Dept. Hygiene, Med. Genet., Shinshu Univ. Sch. Med., Matsumoto)

Chromosomal instability is known to be associated with certain cancer-prone disorders such as Fanconi's anemia. These disorders, designated DNA repair syndromes, have common characteristics such as autosomal recessive inheritance mode, short stature and mental retardation. MEN1 is an autosomal dominant disorder characterized by development of tumors or hyperplasia in two or more endocrine organs, and chromosomal instability in patients with MEN1 has been described. The clinical features of MEN1 are, however, distinct from other DNA repair syndromes except predisposition to tumors. Therefore, we reevaluated chromosomal instability in familial and sporadic MEN1 patients and their relatives including an asymptomatic carrier confirmed by linkage study. Phytohemagglutinin stimulated lymphocytes were cultured with and without diepoxybutane (DEB), and chromosome aberrations were analyzed in Giemsa stained preparations. An increase in the frequency of spontaneous chromosomal aberrations in some patients with MEN1 was observed. With DEB, we found a significant increase in chromosomal aberrations in MEN1 patients and an asymptomatic carrier, but not in control subjects. We conclude that chromosomal instability exists in patients with MEN1 and an asymptomatic carrier of it. Molecular mechanisms of this phenomenon and relationship to tumorigenesis in endocrine organs should be elucidated.

A-62

ANALYSIS OF THE DNA SPOTS SPECIFIC FOR A LUNG TUMOR IDENTIFIED BY TWO-DIMENSIONAL ELECTROPHORESIS OF DNA. Mieko KODAIRA¹, Keiko HIYAMA^{1,2}, Masaaki IMANAKA¹, Satomichi KANEOKA¹, Takahiro TSUJI¹, Chiyoko SATOH¹, Michio YAMAKIDO², Jun-ichi ASAKAWA¹ (Dept. Genet. Radiation Effects Research Foundation, Hiroshima, ²2nd Dept. Internal Medicine, Hiroshima University School of Medicine, Hiroshima)

We have developed a vertical two-dimensional electrophoresis (2-DE) in which an agarose disc gel made in teflon-tubing was used. Alterations in a lung tumor was examined by this technique. Genomic DNAs were digested with *NotI* and *EcoRV* and labeled at the *NotI* sites before the first dimensional electrophoresis. The separated DNA in the disc gel was further digested with *HinfI* prior to the second-dimension electrophoresis. When intensities of spots on the 2-DE pattern are quantitated, copy numbers of corresponding DNA fragments can be estimated. The use of *NotI*, a methylation sensitive enzyme, allows studies of genomic DNA methylation. Comparison of the 2-DE patterns of the primary tumor and its surrounding normal tissue from a lung tumor patient revealed DNA spots with increased copy numbers in the tumor. We cloned *NotI/HinfI* DNA fragments which were directly recovered from the 2-DE gel spots and characterized them. One of intensified spots derived from DNA fragments amplified in this tumor. Each of another 4 intensified DNA spots was revealed to be a part of a repeating unit containing a *NotI* recognition sequence. The *NotI* site in each repeating unit was methylated and resistant to be cleaved with *NotI* in the normal cells, but the intensified signals in the tumor were caused due to demethylation in these recognition sequence of *NotI*.

A-63

ANALYSIS OF A CANDIDATE TUMOR SUPPRESSOR GENE FOR OVARIAN CANCER ISOLATED FROM CHROMOSOME 6q27. Syuya SHIRAHAMA, Susumu SAITO (SRL, Inc., Tokyo) and Yusuke NAKAMURA (Lab. Mol. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

The frequent losses of heterozygosity (LOH) observed on chromosome 6q in ovarian carcinomas suggest the presence of one or more putative tumor suppressor genes on this chromosomal arm. We previously observed frequent allelic losses in ovarian tumors between loci at 6q26 and 6q27 and defined a commonly-deleted region between loci defined by Cl6-111 (D6S193) and Cl6-24 (D6S149). From this region we isolated YAC clones and constructed a cosmid contig map. To isolate expressed sequences from this region, we performed exon trapping experiments and we found that the human AF-6 gene, which is disrupted in acute myeloid leukemia cells that carry a (6;11)(q27;q23) translocation, is located within the commonly-deleted region. Subsequent screening of the AF-6 gene in ovarian carcinomas revealed no mutations. Cosmid contig map and expressed sequences will contribute important data for isolating other candidate genes and should facilitate eventual identification of the putative ovarian tumor suppressor(s) on chromosome 6q.

A-64

DETECTION OF GERM-LINE MUTATIONS OF THE ADENOMATOUS POLYPOSIS COLI GENE BY NON-RADIOACTIVE PCR-SSCP. Yoshinori NIMURA¹, Chizumi FURUWATARI², Minoru FUJIMORI¹, Wataru ADACHI¹, Yoshifumi OGISO², Ken-ichi FURIHATA², Tsutomu KATSUYAMA², Jun AMANO¹ (1 Department of Surgery and 2 Department of Clinical laboratory, Shinshu University school of medicine, Matsumoto)

Germline mutations of the adenomatous polyposis coli (*APC*) gene have been reported in patients with familial adenomatous polyposis and believed to be an early events in colorectal carcinoma. We report the results of screening for the germ line mutations of the *APC* gene in 3 cases of 2 kindreds using PCR-SSCP (Polymerase chain reaction-single strand conformation polymorphisms) analysis and DNA sequencing.

The mutation in kindred 1 was 4 bp deletion at codon 849 in exon 15, resulting in a frameshift leading to truncation of the *APC* gene product at 30 bp downstream. In kindred 2, a transition of C to G at codon 2038 was observed, resulting in an amino acid change from leucine to valine. In this case, it is possible to screen presymptomatic diagnosis easily and quickly with digestion by restriction enzyme *Eco*NI.

A-65

ANALYSIS OF THE WERNER SYNDROME GENE MUTATIONS. Atsuyuki MORISHIMA, Jun NAKURA, Lin YE, Tetsuro MIKI, Toshio OGIHARA (Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka), Junko OSHIMA, GM MARTIN (Dept. Pathology, Univ. of Washington, Seattle), and Chang-En YU, GD SCHELLENBERG, (Dept. Neurology, Univ. of Washington, Seattle)

Werner syndrome (WRN) is a rare autosomal recessive disorder, one of the progeroid syndromes. This syndrome is a model of aging, and characterized by features of premature aging : cataracta, hypogonadism, osteoporosis, and hyperkeratosis. The mean age-at-death is about 50 years old, and main cause of death are neoplasias, myocardial infarcts, and cerebral vascular disease.

The gene for WRN, located on chromosome 8p12, was recently isolated by positional cloning methods. The WRN gene encodes a 1432 amino acid protein, which shows homology to the DExH box DNA and RNA helicases.

Four mutations has been detected in this gene. We have studied these mutations by SSCP method on Japanese population . In the result, one particular mutation holds about 70% of the WRN gene mutations in Japanese.

A-66

A GENETIC, PHYSICAL, AND EST MAP AROUND THE WERNER'S SYNDROME REGION (8p12-21). Lin YE, Jun NAKURA, Atsuyuki MORISHIMA, Noriaki MITSUDA, Tetsuro MIKI and Toshio OGIHARA (Dept. Geriatr. Medicine, Osaka University, Osaka)

Werner syndrome (WS) is an autosomal recessive disorder characterized by the premature occurrence of many age-related features. To identify the gene for WS (WRN), previously assigned to the 8p12-21 region, we used the positional cloning strategy. First, we constructed a genetic map. The map comprises 34 dinucleotide repeat polymorphic markers and spans about 43 cM across the 8p12-21 region. Second, we analyzed 58 patients for the 34 markers by homozygosity analysis to refine the WRN locus, resulting in narrowing the position of the WRN locus to 1.6 cM. Third, we tested linkage disequilibrium between the WRN locus and 16 dinucleotide repeat polymorphic marker loci positioned within the WRN candidate region. This resulted in detection of linkage disequilibrium between WRN and D8S1223 ($p < 0.05$), D8S1055 ($p < 0.01$), D8S1445 ($p < 0.001$), D8S339 ($p < 0.001$), GSR1 ($p < 0.001$), GSR2 ($p < 0.001$), and D8S1444 ($p < 0.05$). Fourth, to construct a physical map containing all of these 7 markers, we assembled 16 YAC clones and 44 P1 clones into a single contig covering a minimum of 1.5 Mb physically and 2.8 cM genetically with 12 polymorphic and 28 nonpolymorphic STSs within the 8p12-21 region. These YAC and P1 clones were used to isolate several candidate genes. Fifth, we also construct a EST map on this cloned region and integrated the EST map into a genetic and physical map. This resulting map should help to promote our understanding of this region.

A-67

THE MUTATIONS OF THE RET PROTO-ONCOGENE WITH MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 (MEN 2). Kenji OGURA, Shuya SHIRAHAMA, Kazumasa HIKIJI (SRL, Inc., Tokyo) Hiroshi TAKAMI (1st Dept. Surg., Teikyo Univ. Sch. Med., Tokyo) Tohichi TOHSEN (Tohsen Clinic, Shizuopka) Kunihiko ITOH (Ito Hospital, Tokyo) and Yusuke NAKAMURA (Lab. Mol. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

Multiple endocrine neoplasia types 2A and 2B (MEN 2A and 2B) and familial medullary thyroid carcinoma (FMTC) are autosomal, dominantly inherited syndromes involving endocrine tumors. Responsible genes of these diseases have been elucidated to be RET proto-oncogene located on chromosome 10q11.2, and it has been shown that, mutations in MEN 2A and FMTC occur at codons 609, 611, 618, 620, 630 and 634 on exons 10 and 11 which encode cystein and in MEN 2B occur at codon 918 on exon 16. We have further studied genetic analysis of RET gene, and found the high frequencis of mutations such as 97%(28/29), 100%(4/4) and 100%(3/3) in 29 cases of MEN 2A, 4 cases of MEN 2B and 3 cases of FMTC, respectively. Furthermore, we identified the mutation of GAG (Glu) to GAC (Asp) at codon 768 on exon 13 in 2 patients of MTC. It was suggested that this genetic analysis of RET proto-oncogene is useful for the screening of carriers having mutations relating to MEN 2A, 2B and FMTC.

A-68

UVB-induced skin carcinogenesis in XPA-deficient mice. Kiyoji Tanaka (Institute for Molecular and Cellular Biology, Osaka University, Osaka)

Nucleotide excision repair (NER) is a versatile repair system capable of rectifying a broad spectrum of DNA damage. Xeroderma pigmentosum (XP), a human inherited disease which is characterized by a high incidence of skin cancer in sun-exposed areas and several neurological complications, has a defect in early steps of NER processes. There are seven different complementation groups (A through G) in XP. We have been interested in the functions of the XPA gene in NER processes. The XPA gene encodes a protein of 273 amino acids with C4 type zinc finger motif, and it preferentially bound to DNA damaged by UV, cisplatin or osmium tetroxide, indicating that the XPA protein is involved in the damage recognition step of NER processes. Besides this DNA binding domain, the XPA protein has the regions involved in the protein-protein interactions to coordinate the NER processes. We found that RPA (replication protein A), ERCC1 protein and several new other proteins bound to the XPA protein. To elucidate the molecular basis of group A XP, we established XPA-deficient mice by gene targeting. The XPA-deficient mice were defective in NER process and highly susceptible to UV-induced skin tumorigenesis. The XPA-deficient mice provide a good in vivo model to study UV-induced skin carcinogenesis in group A XP patients.

A-69

Characterisation of germline mutations in the neurofibromatosis 1 (NF1) gene in Japan.

Nobuaki HATTA, Yuzuru KOBAYASHI, Shigeru FUJITA (The First Dept. Int. Med., Ehime Univ., Ehime)

Takahiko HORIUCHI (The First Dept. Int. Med., Kyushu Univ., Fukuoka)

Neurofibromatosis 1 (NF1) is an autosomal dominant disease characterized by abnormalities in multiple tissues derived from the neural crest. The gene responsible for NF1 was estimated recently. We analysed 95 unrelated Japanese patients for NF1 gene mutation by PCR/SSCP analysis for all exons. Here we demonstrated eleven deletions, three insertions, three missense mutations, three nonsense mutations and three splicing mutations. And also, we employed cDNA analysis such as RT-PCR and RT-PCR/SSCP, and detected four splicing mutations with exon skipings in addition to the above mutations. Most of the detected mutations generated frameshifts or stop codons resulting in truncation of NF1 product (neurofibromin). Those results suggested that our strategies such as PCR/SSCP and RT-PCR/SSCP were useful tools for the investigation of NF1 mutations.

A-70

MUTATIONS IN THE *BRCA2* GENE IN PRIMARY BREAST CANCERS AND OTHER CANCERS. Toyomasa KATAGIRI, Maiko MINAMI, Yumi NAKAJIMA, Hiroko BANDO, Yoshio MIKI (Dept. Hum. Genome Anal., Cancer Chemother. Ctr., Jpn. Fdn. Cancer Res., Tokyo) and Yusuke NAKAMURA (Dept. Hum. Genome Anal., Cancer Chemother. Ctr., Jpn. Fdn. Cancer Res. and Lab. Mol. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

To define the role of *BRCA2* in sporadic breast cancer and other cancers, we screened the entire coding region of this gene for mutations in 100 primary breast cancers, 60 hepatocellular carcinomas (HCCs), and 36 pancreatic cancers (PCs) from Japanese patients. We found three mutations including two germline and one somatic mutations in primary breast cancers. One of the germline mutations was insertion of *Alu* element into exon 22, and the other showed 4-bp deletion in exon 9. Somatic mutation was a missense mutation of unknown significance. No alteration was found in any of PCs examined. Three mutations were identified in HCCs; one was a somatic 6-bp deletion within intron 6. The other two mutations were missense mutations in the germline. Furthermore, we found that *BRCA2* expressed in adult liver tissue. These results indicate that somatic mutations in *BRCA2* is very rare, like somatic mutations in *BRCA1*, in primary breast cancer, and inactivation of *BRCA2* may play some role in development or progression of HCC.

A-71

ANALYSIS OF p16 AND p15 GENE ALTERATION IN ORAL SQUAMOUS CELL CARCINOMA CELL LINES, Narikazu UZAWA^{1,2}, Daisuke AKANUMA^{1,2}, Mitsuaki A. YOSHIDA¹, Teruo AMAGASA², Masao S. SASAKI³ and Tatsuro IKEUCHI¹. (¹Dept. Cytogenet., Med. Res. Inst., ²1st Dept. Oral & Maxillofac. Surg., Fac. Dent., Tokyo Med. & Dent. Univ., Tokyo., ³Radiation Biology Center, Kyoto Univ., Kyoto)

Allelic loss at 9p21 occurs frequently as an early event in head and neck squamous cell carcinoma(SCC) including oral cancer. The *p16/MTS1/CDKN2A* and *p15/MTS2/CDKN2B* genes, which code cell cycle regulator proteins, have been mapped to the 9p21 region. Highly frequent alterations of these genes have been reported in human cancers. However, whether *p16* and/or *p15* inactivations are the target of this early loss event in oral SCC has remained unsolved. We examined the genomic status and mRNA expressions of these genes in 11 oral SCC cell lines by using methods based on PCR. Homozygous deletion of both *p15* and *p16* was detected in only one cell line. RT-PCR analysis revealed that two and five cell lines did not express *p16* mRNA and *p15* mRNA, respectively. We are now investigating these gene alterations including deletion, abnormal methylation and mutation, in primary samples of oral SCC.

A-72

ISOLATION OF cDNA CLONES FROM A BREAKPOINT OF INV(21) IN A PATIENT WITH TRANSIENT ABNORMAL MYELOPOIESIS (TAM)

Motoi NAKANO, Tohru OHTA, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

TAM is a transient leukemia occurring occasionally in Down syndrome newborn infants. We presumed the TAM locus at 21q11.1, based on the results of our cytogenetic and molecular studies. We previously encountered a TAM patient with inv(21)(q11.1q22.13). We confirmed that the proximal breakpoint of the inv(21) of the patient is located between two STS markers at 21q11.1, and isolated a cosmid clone (Q62E11) corresponding to the breakpoint.

In the present study, we have isolated several cDNA clones from the cosmid, Q62E11. In order to know that the sequence in this clone was conserved in other animals, zoo-blotting was first performed. A 15-kb *EcoRI* subclone detected hybridization signals in cow, pig and other mammals. As a CpG island was found in this fragment, we screened an adult bone-marrow cDNA library and isolated 13 cDNA clones. We are characterizing these cDNA clones.

A-73

GENE THERAPY FOR PHENYLKETONURIA USING REPLICATION-DEFECTIVE RECOMBINANT ADENOVIRUS. Yutaka NAGASAKI, Yoichi MATSUBARA, Kunihiro FUJII, Jun AKANUMA, Hideaki TAKANO, Shigeo KURE, Kazutosi TAKAHASHI, Kuniaki NARISAWA (Dept. Biochem. Genet., Tohoku Univ. Sch. Med., Sendai) and Yumi KANEGAE, Izumu SAITO (Lab. Mol. Genet., Med. Sci. Inst., Tokyo Univ., Tokyo)

Phenylketonuria (PKU) is due to deficiency of phenylalanine hydroxylase (PAH). It causes severe mental retardation, hypopigmentation and hyperphenylalanemia. Toward the gene therapy of PKU, we constructed a replication-defective recombinant adenovirus using a cosmid-cassette method. The adenovirus was administered into tail veins of PKU model mice. Serum phenylalanine level decreased to less than 10% (2.2mg/dl) of the pre-treatment value (30.9mg/dl) within 24 hrs and lasted for 10 days. Daily subcutaneous injection of immunosuppressant FK506 after adenovirus injection prolonged the period to more than 35 days and allowed successful repeated gene delivery. PAHcDNA was mainly detected in liver. PAH activity was present exclusively in liver. In these experiments, hypopigmented PKU mice showed pigmentation of hair, from grayish color to black. The study demonstrated the correction of physical phenotype as well as biochemical phenotype by gene transfer and efficacy of immunosuppressant for adenovirus-mediated gene therapy.

A-74

EXPRESSION OF MULTIPOTENTIAL NEURAL STEM CELL MARKERS IN AMNIOTIC EPITHELIAL CELLS FROM HUMAN, MONKEY AND RAT. Norio SAKURAGAWA, Ramasamy THANGAVEL (Dep. Inher. Met. Dis., Nat. Inst. Neurosci., NCNP, Tokyo), Osamu OKUDA, Ikuko OGINO (Dep. Neurosug. Juntendo Univ. Sch. Med., Tokyo), Fumiko ONO, Yasuhiro YOSHIKAWA (NIH: Tsukuba Primates Center, Ibaragi)

Human amniotic epithelial cells (AEC) are formed from amnioblasts, separated from the epiblasts at about the 8th day after fertilization. Recently we found the expression of markers for both neuronal and glial cells in human amniotic epithelial cells. In this study we report the comparative study using AEC from human, monkey and rat. AEC were immunocytochemically stained as described previously. Appropriate FITC- or rhodamin-labeled antibodies were used for double staining. AEC from human, monkey and rat displayed positive immunoreactivity to neural stem cell markers as well as neural and glial cell markers. Double staining for neuron and glial cells showed coexpression of these markers in the majority of cells. In addition, oligodendrocyte cell lineage was present in the same field as well as cortical glial cell lineage, indicating heterogeneous coexpressions. These results showed that expression of neural stem cell markers is present in the different species, indicating possible use of animal models of transplantation of these cells into brain for treatment of neurodegenerative or metabolic diseases.

A-75

THE RECOMBINANT ADENO-ASSOCIATED VIRUS FOR HEPATIC GENE THERAPY. Torayuki OKUYAMA, and Masao YAMADA. (Dept. Genet., National Children's Medical Research Center, Tokyo)

We synthesized recombinant adeno-associated virus vector for hepatic gene therapy. The construct pTRNAET was made to clone a 1.7 kb Neo^R cassette (PGK promoter-Neo^Rgene-rabbit β -globin polyA) and a 3.5 kb liver specific expression cassette for hAAT (ApoE enhancer -hAAT promoter-hAATcDNA-SV40polyA) into an AAV vector plasmid, pTR(+). We established the stable clone HeLa-TRNAET which carries the DNA of pTRNAET in HeLa cells. The plasmid pIM45 (packaging plasmid for AAV) and Ad5 was transinfected into HeLa-TRNAET to synthesize the rAAV vector AAV-TRNAET. The Neo^R titer of AAV-TRNAET is approximately 1.0×10^5 cfu / p100 in the examined four cell lines (HeLa, HepG2, NIH3T3, and Hepa1A). We detected the expression of hAAT in gene-transduced Hepa1A cells, and the level of expression corresponded 25% of endogenous level of hAAT in HepG2 cells. However, no expression was seen in the HeLa cells transduced by the rAAV. Based on these observations, we conclude that the AAV-TRNAET can transduce the cell lines derived from hepatocytes efficiently and keeps the tissue specificity in expression.

B-1

AN ASSOCIATION OF DRB1*1501 WITH WESTERN TYPE MULTIPLE SCLEROSIS IN JAPANESE. Yasuharu NISHIMURA¹, Takayuki KANAI¹, Jun-ichi KIRA² and Takuro KOBAYASHI² (¹Div. Immunogenet., Kumamoto Univ. Grad. Sch. Med. Sci., Kumamoto, ²Dept. of Neurol., Neurol. Inst., Faculty Med., Kyushu Univ., Fukuoka)

In caucasians, the statistically strong association between HLA-DRB1*1501 and multiple sclerosis (MS) was well established. Whereas it is controversial in Japanese patients with MS. In this study the polymorphism of HLA-DRB1, -DRB3 and -DRB5 genes as well as magnetic resonance imaging (MRI) of the brain and spinal cord were studied in 57 Japanese patients with MS. According to the clinical findings, 23 patients who showed a relapsing-remitting course and a selective involvement of the optic nerve and spinal cord with minimal brainstem signs were classified as Asian type MS. The other 34 patients demonstrating an involvement of multiple sites in the central nervous system were classified as Western type MS. Asian type MS showed a significantly lower number of brain lesions on MRI than did Western type, while Asian type MS showed a significantly higher frequency of gadolinium-enhanced lesions on the spinal cord MRI than did Western type (47% vs. 17%). Among the DRB chain genes examined, only the frequency of the DR2-associated DRB1*1501 allele and DRB5*0101 allele were found to be significantly higher in Western type (41.2%) than in either Asian type (0%) or healthy controls (14.2%). Heterogeneity in the immunogenetic background and in the MRI features between the two subtypes of MS thus suggests the presence of two etiologically distinct MS in Asians.

B-2

IDENTIFICATION OF AN AUTOIMMUNE T CELL EPITOPE PRESENTED BY THE DISEASE SUSCEPTIBLE HLA CLASS II IN INFANT-ONSET MYASTHENIA GRAVIS. Takayuki KANAI¹, Yoshiko NOMURA², Masaya SEGAWA², Sho MATSUSHITA¹ and Yasuharu NISHIMURA¹(¹Div. Immunogenet., Kumamoto Univ. Grad. Sch. Med. Sci., Kumamoto, ²Segawa Neurological Clinic for Children, Tokyo)

Infant-onset (<3y) myasthenia gravis (MG) is an Orientals-specific autoimmune disease that affects predominantly neuromuscular junctions in ocular muscles. It was reported that the frequency of Orientals-specific HLA class II alleles, HLA-DR9-DQ9, DR13-DQ6 and HLA-DR9/DR13 heterozygote were significantly increased in the patients. We synthesized overlapping peptides covering acetylcholine receptor α -subunit (AChR α), regarded as an autoantigen in MG. By stimulating peripheral blood mononuclear cell (PBMC) from a patient heterozygous for HLA-DR9-DQ9 and DR13-DQ6 with a mixture of those overlapping peptides, we established an AChR α peptide (p71-91) specific autoreactive CD4⁺ $\alpha\beta$ T cell clone. The T cell clone recognized the AChR α peptide in the context of HLA-DQ6 molecule. A core epitope of the autoantigenic peptide is encoded for by an alternatively spliced exon P3A of AChR α gene. The T cell clone produced a large amount of IFN- γ , whereas produced little IL-4, suggesting Th1 like phenotype. The result seems to be a proof that the level of anti-AChR autoantibodies in serum with infant-onset MG patients is lower than adult-onset MG in which Th2 like AChR autoreactive T cells dominate to produce a large amount of anti-AChR autoantibody. Thus, an orientals-specific HLA class II allele directly controls susceptibility to infant-onset MG, specific to Orientals.

B-3

FUNCTIONAL EXPRESSION OF HUMAN INTERLEUKIN-4 RECEPTOR α GENE ON A MOUSE MACROPHAGE CELL LINE. Takeshi OTSUKA, Yoshiaki NEMOTO, Kunihiro YAMAOKA, Hitoshi NAKASHIMA, Hiroaki NIRO, Yosuke TANAKA, Eiichi OHGAMI, Yojiro ARINOBU, Yoshiyuki NIHO (Dept. Int. Med., Kyushu University, Fukuoka) and IZUHARA Kenji (Dept. Hum. Genetics, National Institute of Genetics, Mishima)

By using B cells of X-linked severe combined immunodeficiency (XSCID) patients, we indicated that mutation of the IL-2 receptor γ chain (γ c) caused abnormal signal transduction not only of IL-2 but also of IL-4. In this study, we evaluated a functional significance of γ c in IL-4 receptor system of monocytes/macrophages. Mouse monocytic cells never responded to human IL-4. We introduced human IL-4 receptor α chain (IL-4R α) gene into a mouse monocytic cell line and established cell lines stably expressing human IL-4R α . Human IL-4, as well as mouse IL-4, reduced productions of TNF- α , prostaglandins and nitric oxide inducibly produced by such transformed cell lines stimulated with lipopolysaccharide. Neutralizing antibody against γ c hardly blocked these functions of human IL-4, indicating that γ c may be less important in transducing signals carried out by human IL-4. Further studies are still needed to explain significance of IL-4R α and γ c of functional IL-4 receptor.

B-4

The Human Gene Encoding The Heavy Chain of The Major Histocompatibility Complex Class I-Like Fc Receptor (*FCGRT*) Maps to 19q13.3

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FcRn is an Fc receptor that structurally resembles the major histocompatibility complex class I molecule. It has been reported that FcRn in the intestinal epithelium of neonatal mice and rats mediates the selective uptake of IgG, and helps to acquire passive immunity. In this study, we isolated the human gene encoding the heavy chain of FcRn (*FCGRT*), and using two *FCGRT* lambda clones H6-1-1 and H9-1-1 (insert sizes of 18 and 16 kb) we mapped it by fluorescence *in situ* hybridization to human chromosome, 19q13.3. Thus, like its mouse counterpart, the human *FCGRT* gene is located outside the major histocompatibility complex.

B-5

HLA SUSCEPTIBILITY GENES IN JUVENILE RHEUMATOID ARTHRITIS.

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HLA class I and class II genes in 66 Japanese patients with juvenile rheumatoid arthritis (JRA) were studied by means of both serological and DNA typing. The typing results revealed positive associations of polyarticular JRA with HLA-A*2402, -B59, -Cw7, and -DRB1*0405-DQB1*0401, pauciarticular JRA with HLA-A*2603 and -B39, and systemic JRA with HLA-A31, -Cw9, -DRB1*1501-DQB1*0602 and -DPB1*0301. These HLA alleles/loci were considered to show independent associations except for the B59-DRB1*0405-DQB1*0401 and DRB1*1501-DQB1*0602 haplotypes. Polyarticular and systemic JRA showed negative associations with DQA1*0501 and the DRB1*0901-DQB1*0303 haplotype, respectively. In particular, the common Japanese haplotype A33-B44-DRB1*1302-DQB1*0604-DPB1*0401 was not detected in any of the JRA patients. These results suggest that 1) the pathogenetic bases of these three JRA subtypes differ and 2) the gene(s) carried by the A33-B44-DRB1*1302 haplotype are protective for all the JRA subtypes.

B-6

INCIDENCE OF HLA-DPB1 AND -DRB1 ALLELES IN PATIENTS WITH KAWASAKI SYNDROME. Michiko ONIMARU, Jun ABE, Takao KOHSAKA (National Children's Med. Res. Ctr., Tokyo), Yoshihisa WATANABE, Satoshi MORIYAMA (JRC Central Blood Center, Tokyo) and Katsushi TOKUNAGA (Dept. Human Genetics, Tokyo Univ.)

Kawasaki syndrome (KS) is an acute vasculitis primarily affecting infants and young children. Although the etiology of the illness is unknown, epidemiological studies indicate that the incidence of KS is especially high in Japan. In this study, we examined the incidence of HLA-DPB1 and -DRB1 alleles among 64 patients with KS using PCR-PHFA (preferential homoduplex formation assay) and PCR-MPH (micro titer hybridization) method, respectively. The results were analyzed by the χ^2 test using the data from Akaza T. et al (Kon-nichi No Ishoku 7, 1994) as a control.

Among the 14 DPB1 alleles studied, no allele had significant difference in the incidence between the patients and controls. On the other hand, the incidence of DR8 was significantly increased in KS. By high resolution typing, 16 out of 64 patients (25%) were positive for DRB1*0803 ($p < 0.05$) and 23 (36%) were positive for either *0802 or *0803 ($p < 0.02$). Between the *0803 positive and negative patients, no significant difference was observed in the patients' age, sex, severity of clinical symptoms, and the laboratory data such as WBC, CRP and IL-6. The incidence of coronary aneurysm was 30.0% and 26.3% in the *0803 positive and negative patients, respectively.

The frequency of DR8 in Japan (13.3%) is the highest compared with the other countries (The 11th International Histocompatibility Workshop, 1991). It might be of interest to determine whether DR8 is also increased in patients from other countries.

B-7

HLA CLASS II DNA TYPING IN BUERGER'S DISEASE. Wulin AERBAJINAI, Terumasa TSUCHIYA, Yukio YASUKOCHI (Dept. Molecul. Genet., M.R.I., Tokyo Med.& Den. Univ., Tokyo.) and Fujio NUMANO (Dept. Int. Med., Tokyo Med.& Den. Univ., Tokyo.)

To analyze the genetic factors involved in the pathogenesis of Buerger's disease, the polymorphisms of HLA class II genes (HLA-DP, DQ and DRB) were investigated in 36 patients using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. HLA class I (B54 and B52) and HLA class II (DQ alleles) genes were also typed by polymerase chain reaction-sequence-specific oligonucleotide probe (PCR-SSOP) method to confirm alleles. PCR-RFLP typing revealed that the frequencies of HLA class II genes, DRB1*0405, DQB1*0401, DQA1*03 and DPB1*0501, were significantly increased in the patients with Buerger's disease compared with the healthy controls. Further, DNA typing confirmed that HLA-B54 was associated with Buerger's disease. These results suggested that the haplotype of HLA-B54-DRB1*0405-DQB1*0401-DQA1*03-DPB1*0501 was associated with Buerger's disease in Japanese population.

B-8

SEQUENCE-LEVEL POLYMORPHISM OF HLA CLASS I GENES (A, B, and C) IN JAPANESE. Katsushi TOKUNAGA^{1,2}, Yoshihide ISHIKAWA², Atsuko OGAWA², Huiru WANG², Makoto BANNAI³, Kenji TADOKORO², and Takeo JUJI² (¹Dept. Human Genetics, University of Tokyo, Tokyo, ²Japanese Red Cross Central Blood Center, Tokyo, ³Tokyo Metropolitan Blood Center, Tokyo)

Nucleotide sequences of HLA class I alleles have been determined in Japanese. Up to now, 23, 42, and 16 different alleles, in which 8, 12, and 3 were new, have been identified at HLA-A, -B, and -C loci, respectively. Thus the HLA diversity at the sequence level is much larger than that detected by conventional serological typing. Moreover, we have performed high resolution DNA typing for these class I genes in a Japanese population sample. Numbers of alleles observed at gene frequencies of more than 3% were 8, 13, and 9 at HLA-A, -B, and -C loci, respectively. Putting class I and II data together, a number of significant associations were detected among different HLA loci, and several common multi-locus haplotypes could be deduced, such as A*3303-Cw*1403-B*4403-DRB1*1302-DQB1*0604 and A*2402-Cw*1202-B*5201-DRB1*1502-DQB1*0601. These findings should become useful for studies on disease susceptibility genes, matching for bone marrow transplantation, and ethnic diversity.

B-9

Chromosomal localization of the human natural killer cell inhibitory receptor family genes and the natural killer gene complex (NKC) by fluorescence *in situ* hybridization. Yumiko SUTO¹, Katsumi MAENAKA¹, Toshio YABE¹, Momoki HIRAI², Katsushi TOKUNAGA^{1, 3}, Kenji TADOKORO¹ and Takeo JUJI¹. (¹JRC Ctrl Bld Ctr, ²Dept Biol Sci, Univ Tokyo, ³Dept Hum Genet, Univ Tokyo, Tokyo)

Recently cDNAs of at least ten human natural killer (NK) cell inhibitory receptor (KIR) family genes have been described. These genes encode transmembrane proteins with two or three immunoglobulin-superfamily extracellular domains. While, in the mouse, a supergene family of type II integral membrane protein with a C-type lectin domain is identified as NK cell receptors and mapped on mouse chromosome 6 region named the NK gene complex (NKC). In this study, the KIR genes (NKAT1, 2) and the human NK-related lectin genes (NKG2A-D, CD69, etc.) were assigned on human chromosomes by modified fluorescence *in situ* hybridization (FISH). Biotin-labeled PCR products under 1 kb were used as probes. Hybridization signals were amplified using rabbit anti-biotin IgG, FITC-goat anti-rabbit IgG, and as the third layer, Cy2-donkey anti-goat IgG. The KIR genes were localized on human chromosome 19q13.4. Detected signals were disproportionately large for the size of the probes, suggesting a tandem arrangement of many genes of this gene family. All the lectin genes examined were localized on human chromosome 12p13, suggesting the presence of the human NKC in this region.

B-10

COMPLEMENTATION STUDY AND GENETIC MAPPING OF NIJMEGEN BREAKAGE SYNDROME

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Nijmegen breakage syndrome (NBS) is an autosomal recessive disease characterized by immunodeficiency, a high incidence of cancer, and hypersensitivity to ionizing radiation. Two complementation groups, V1 and V2, have been reported in NBS, based on restoration of RDS. We have newly established V1 cell lines using SV40 transformation. To localize the NBS gene, we have performed functional complementation assays using somatic cell fusion between NBS-V1 and NBS-V2, and then carried out a genome-wide search for NBS locus using microcell-mediated chromosome transfer, followed by complementation assays based on radiosensitivity. We found that radiation resistance was not restored in fused cells between NBS-V1 and NBS-V2. Only human chromosome 8 complements the sensitivity to ionizing radiation in NBS cell lines. Merely the long arm of chromosome 8 was sufficient for restoring the defect. Our results suggested that NBS is a homogeneous disorder, and that the gene for NBS is located at 8q.

B-11

WISKOTT-ALDRICH SYNDROME IN TWO SISTERS. Tatsuro KONDOH, Tadashi MATSUMOTO, Yoshiro TSUJI (Dept. Pediatrics, Nagasaki University School of Medicine, Nagasaki)

We report two sisters in a family representing manifestations of Wiskott-Aldrich syndrome (WAS). The younger sister had suffered from recurrent infections, small thrombocytopenic petechiae, purpura, and eczema, and died of intracranial bleeding at age 2 years. The elder sister had the same manifestations as the younger sister's. She took Takayasu disease from 8 years old, and died of the rupture of the aorta at age 10 years. All the laboratory data of them were compatible with WAS. RT-PCR study against WASP mRNA in the elder sister's peripheral leukocytes could not be shown the abnormal sized fragment. Studies on the mother-daughter transmission of X chromosome suggested that each sister had inherited a different X chromosome from the mother. Sialophorin analysis revealed that in the elder sister a 115-KD band that should be specific for sialophorin was reduced in quantity, and instead an additional 135-KD fragment was present as a main band. PCR and SSCP analysis of the sialophorin gene demonstrated that there were no detectable size-change nor electrophoretic mobility change. Including mother-daughter transmission tests of chromosome 16, the results indicated that their sialophorin gene structure should be normal.

Recently, it is reported that WAS protein is a novel effector for the GTPase CDC42Hs. At least two explanations are plausible for the occurrence of the WAS in our cases : our patients have an autosomal gene mutation which break the CDC42Hs-WASP pathway, or, alternatively, the condition in this family is an autosomal recessive disorder separated etiologically from the X-linked WAS.

B-12

BONE MARROW TRANSPLANTATION IN DI GEORGE SYNDROME. Masahiko NAKAYAMA, Tatsuro KONDOH, Toshimitsu TAKAYANAGI, Tadashi MATSUMOTO, Yoshiro TSUJI (Dept. Pediatrics, Nagasaki University School of Medicine, Nagasaki)

Di George syndrome is a congenital immunodeficiency associated with defective development of the first through sixth branchial pouches, manifested clinically by peculiar facies, hypoparathyroidism and hypocalcemia, congenital heart disease, and increased susceptibility to infections due to the deficiency of T cell function by a/hypo-plasia of the thymus. As one of treatments, there are two reports that bone marrow transplantation (BMT) is effective to Di George syndrome patient. We report a severe type of Di George syndrome patient who was treated by BMT. The patient was the 3275g full-term, appropriate for gestational age male, born to unrelated parents. At birth the infant had cyanosis, and diagnosed as the severe type of Tetralogy of Fallot by cardioechogram and cardiac catheterization. We tried chromosomal analyses twice to him, but his lymphocytes did not respond to PHA stimulation. He has clinical features consistent with Di George syndrome including peculiar face, hypocalcemia due to hypoparathyroidism, and reduction of the percentage of CD3, CD4 and CD8 in his peripheral lymphocytes. FISH study with the chromoprobe located on CATCH 22 region showed the only one signal suggested the deletion in this region on one allele. The tendency of susceptibility to infections and his general conditions become worse. So we had to choose BMT therapy to him, because thymic transplantation method could not be available actually. As a conditioning and a prevention for GVHD, ATG and CyA/MiX were used, respectively. We checked whether BMT to him was succeeded or not by DNA analysis, but the result was a rejection. So, we proceed to re-BMT now.

B-13

LARGE-SCALE CHROMOSOMAL MAPPING OF HUMAN cDNA BY FISH. Masami NAGATA, Mikio SUZUKI, Ako TAKAICHI, Hiroichi SHINOMIYA, Takeshi WATANABE, Tsutomu FUJIWARA, Ei-ichi TAKAHASHI, (Otsuka GEN Res. Inst., Otsuka Pharmaceutical Co. Ltd., Tokushima) and Yusuke NAKAMURA (Lab. of Molecular Medicine, Inst. of Medical Science, The Univ. of Tokyo, Tokyo)

We have performed a single pass sequencing from fetal brain, aorta and placenta cDNA libraries to isolate novel genes responsible for genetic diseases and cancers. Chromosomal mapping is an essential step for positional candidate approach from these random cDNA sequences.

We have constructed an arrayed human genomic cosmid and BAC library, and prepared high density replica filters for colony-hybridization and DNA pools for PCR screening. Using this system, a total of 481 clones corresponding to cDNAs have been isolated and mapped by direct R-banding FISH. Among these clones, about 90% were preferentially sublocalized to R-positive bands, with the half of these to T-band regions. These results indicate that human genes are unequally distributed on chromosomal bands.

B-14

ASSIGNMENT OF THE HUMAN *GLI2* GENE, *GLI2*, TO 21q14 BY FLUORESCENCE IN SITU HYBRIDIZATION

Osamu MIYOSHI, Naomichi MATSUMOTO, Masahiro FUJIMOTO, Rumiko KATO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

GLI2 belongs to a gene family including the human oncogene, *GLI*, and its related gene, *GLI3*. Disruption of *GLI3* in Greig cephalopolysyndactyly syndrome (GCPS) patients suggested that this gene plays a role in limb/craniofacial development in mammals. *GLI2* was localized to chromosome 2 by a human-rodent hybrid panel analysis. The present study deals with subregional mapping of *GLI2* by FISH. A bacterial artificial chromosome (BAC) clone, 28F13, containing *GLI2*, was isolated from a human BAC library by means of PCR. A set of primers for PCR was designed from the genomic *GLI2* sequences. The BAC-DNA was used as a FISH probe on human R-banded metaphase chromosomes. The signals were clustered on chromosome band 2q14, and no specific double-signals were observed on any other chromosomal regions, thus allowing the precise localization of *GLI2* to 2q14. The predicted amino acid sequences of the zinc finger regions show 89% similarity between *GLI2* and *GLI*, and 92% between *GLI2* and *GLI3*. The *GLI*-family probably encodes DNA- or RNA-binding proteins which may regulate transcription and involves an important biological process. Since a *GLI3* disruption results in GCPS, it is plausible that *GLI2* also plays a role in the development in humans. Although no disease loci reminiscent of developmental defects have been assigned to 2q14, once mapped to this region, patients with such a disorder may represent a *GLI2* mutation.

B-15

ASSIGNMENT OF THE HUMAN CONNEXIN43 GENE, *GJA1*, TO 6q22.3

Rumiko KATO, Naomichi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Connexin43 is one of connexin proteins which make up the intercellular gap junctions, and are abundantly expressed in the mammalian heart. Targeted null mutation of the mouse connexin43 gene has been reported to be neonatally lethal due to right ventricular outlet stenosis. Moreover, single-base mutations of the human homolog (*GJA1*) were identified in patients with laterality defects of the chest and abdominal organs, suggesting that connexin43 is participating in embryogenesis, cellular differentiation, and development of heart. The locus of an expressed human connexin43 gene (*GJA1*) has been sublocalized to band 6q21-q23.2 by a study with somatic cell hybrid panel. We isolated a bacterial artificial chromosome (BAC) clone that covered almost the entire *GJA1*. Then, fluorescence *in situ* hybridization (FISH) using the BAC clone as a probe, and sequential G-banding on the same metaphases revealed that *GJA1* was assigned to chromosome 6q22.3.

B-16

GENOME ANALYSIS OF HUMAN CHROMOSOME 14Q32 REGION FOR SEARCHING DISEASE RELATED GENES. Masaharu ISOBE, Jun SUGIMOTO, Hiroshi KATO, Toyomasa HATAKEYAMA (Molecular and Cellular Biology Lab., Faculty of Engineering, Toyama University, Toyama)

Band 32 region on the long arm of human chromosome 14 (14q32) contains about 10 Mb of genomic DNA. The region represented by this cytogenetic band is known to contain several genes of biological and medical interest such as the putative oncogene for T-cell leukemia and the tumor suppressor gene(s) for colon and bladder cancer as well as the gene for Usher syndrome type IA. As a first step to identify those disease related genes, we have generated a physical map of the human chromosome 14 at band q32 by using yeast artificial chromosome (YAC) clones. The contig spanning about 8Mb between D14S81 and D14S250 has been assembled by STS and probe content mapping for 40 loci with average spacing of about 200 kb. A subset of YAC clones has been used for searching transcripts from 14q32 region. We have isolated 18 independent cDNA clones by screening cDNA libraries using evolutionally conserved DNA fragments found in those YAC clones as probes. The several fold coverage of 14q32 region and isolated cDNA clones will provide a reliable resource for the searching disease related genes.

B-17

LOCALIZATION OF THE HUMAN CYTOSOLIC ACETOACETYL-COENZYME A THIOLASE (ACAT2) GENE TO CHROMOSOME 6q25.3-q26. Mitsuo MASUNO¹, Toshiyuki FUKAO², Xiang-Qian SONG², Seiji YAMAGUCHI³, Tadao ORII², Naomi KONDO², Takuma ISHII¹, Kiyoshi IMAIZUMI¹, and Yoshikazu KUROKI¹ (¹Div. of Med. Genet., Kanagawa Children's Med. Ctr., Yokohama; ²Dept. of Pediatr., Gifu Univ. School of Med., Gifu; and ³Dept. of Pediatr., Shimane Med. Univ., Izumo)

Previously, we have cloned the human cytosolic acetoacetyl-CoA thiolase (ACAT2; EC.2.3.1.9) cDNA and have found that the 3' portion (330 bp) of the sense strand sequence of the ACAT2 cDNA complementarily overlapped with the 3' region of the human t-complex polypeptide-1 (TCP1) gene. As the human TCP1 gene has been assigned to chromosome 6q25-q27, the ACAT2 gene is considered to be localized to the same chromosome region. To confirm this speculation and determine the location of the ACAT2 gene more precisely, we performed FISH experiments using a cDNA probe(849-bp EcoRI fragment) of the human ACAT2.

Among 117 R-banded (pro)metaphase spreads examined, 40 (34%) exhibited 148 hybridization signals. Twenty-four signals on 17 (pro)metaphase spreads localized to 6q25.3-q26. As the majority of the signals were located on or near the distal end of 6q25.3, with some signals extending into 6q26, the most likely location of the ACAT2 gene is in 6q25.3.

B-18

Precise chromosomal mapping of 280 unidentified human genes by FISH and use of radiation hybrid panels.

Naohiko SEKI, Takahiro NAGASE, Ken-ichi ISHIKAWA and Nobuo NOMURA
(Gene Strucrute I, Kazusa DNA Research Institute, Chiba)

We have isolated a total of 280 unidentified full-length cDNA clones with relatively long sizes from human immature myeloid cell line KG-1 and human brain, and analyzed their sequence feature as well as expression profiles in various human tissues. The chromosomal assignment of these clones has also been made by scoring segregation patterns of PCR products in human-rodent somatic cell-hybrid DNA. We now determined more precise chromosomal location of 280 genes by fluorescence *in situ* hybridization (FISH) or by the use of radiation hybrid panels. The mapping data will provide valuable resources for gene hunting in the human genomic regions where disease-related linkages are suggested or being pursued.

B-19

FINE REGIONAL MAPPING OF HUMAN CHROMOSOME 2q12-q14 WITH 36 COSMID MARKERS BY FISH. Momoki HIRAI, Fumio KASAI, (Dept. Biol. Sci., Tokyo Univ., Tokyo), Yumiko SUTO (Japan Red Cross Blood Ctr.), Kumiko KOYAMA, Yusuke NAKAMURA (Inst. Med. Sci., Tokyo Univ., Tokyo) and Ei-ichi TAKAHASHI (Ohtsuka Gen Inst., Tokushima)

The order of 36 cosmid markers derived from human chromosome 2q12-q14 was exactly determined by high-resolution fluorescence in situ hybridization (FISH). This region includes the telomere-to-telomere fusion point of two ancestral ape-type chromosomes. These clones were hybridized to R-banded chimpanzee chromosomes. Seventeen centromeric clones were located to chimpanzee chromosome 12p, while 19 telomeric clones were located to chimpanzee chromosome 13p. The order of clones in each chimpanzee chromosome was identical to that in human chromosome 2. This high-resolution mapping, therefore, contributes not only to genetic mapping but also to the dating of chromosomal reorganization that might have occurred in primate evolution.

B-20

PARTIAL GENOMIC STRUCTURE OF THE HUMAN DNA-PKcs GENE THAT COMPLEMENTS HYPER-RADIOSENSITIVITY OF THE *SCID* MUTATION. Masahiro FUJIMOTO, Naomichi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki) and Kenshi KOMATSU (Dept. Radiat. Biol., Res. Inst. Radiat. Biol. Med., Hiroshima Univ., Hiroshima)

Murine severe combined immunodeficiency (*scid*) is an autosomal recessive disorder characterized by a lack of functional B and T lymphocytes due to a failure of V(D)J recombination activity and hyper-radiosensitivity due to a failure of DNA double-strand break repair. We previously mapped the putative human gene for hyper-radiosensitivity that is complementary to the murine *scid* to human chromosome 8q11.1 by fluorescence *in situ* hybridization (FISH) using human-*scid* mouse radiation cell hybrids. The human DNA-dependent protein kinase catalytic subunit gene (*DNA-PKcs*) is a strong candidate which complements the *scid* mutation and its huge cDNA (13.4kb) has been cloned. In our previous study we isolated by a CEPH-YAC library screening 3 yeast artificial chromosome (YAC) clones covering *DNA-PKcs*. We subcloned the YACs to cosmid clones containing 5'-*DNA-PKcs*. From the cosmid clone sequences, we identified the first 10 intron-exon boundaries of *DNA-PKcs* with a primer walking strategy. The length of the 10 exons is from 31bp to 206bp and an average length 103bp. The sequences of the 10 exons matched exactly those of the corresponding cDNA. Predicted intron-exon boundaries fit well with the GT-AG rule for splice site selection.

B-21

MOLECULAR ANALYSIS OF THE ENDPOINT OF THE COMMON LARGE DELETION IN CATCH22. Hiroki KURAHASHI^{1,2}, Takahiro NAKAYAMA¹, Shintaro OKADA², and Isamu NISHISHO¹. (1Dept. Med. Genet., 2Dept. Pediatr., Osaka Univ. Med. School., Osaka)

CATCH22 patients are known to show marked phenotypic variability, although the extents of 22q11 deletion are uniform. It is suggested that at both ends of the deletion there lies region specific repetitive sequence prone to deletion. We firstly analyzed YAC y966A8 which should contain the proximal end, resulting that critical region is revealed to be deleted in the YAC. Then, y849E9 which should contain the distal end was subcloned into cosmids and analyzed by FISH and Southern hybridization. Approximately half of the cosmids were single copy and located out of the common large deletion. The other half were found to contain 22q11 specific multicopy sequences. FISH analysis on various translocated chromosomes showed that their homologous regions are located near the proximal end. It is suggested that these 22q11 specific repetitive sequences are related to development of the common large deletion. However, no cosmids showed apparent rearranged bands in patients with deletion. In this region, we identified a VNTR whose homologous region is also located near the proximal end. Detailed analysis of the vicinity of the VNTR revealed that some of patients have rearrangement in the VNTR. It is strongly suggested that this VNTR should be related to development of some types of CATCH22 deletion.

B-22

CONSTRUCTION OF BAC/COSMID CONTIGS FOR THE CAT EYE SYNDROME CHROMOSOME REGION (22q11) Shinsei MINOSHIMA, Shuichi ASAKAWA, Kazuhiko KAWASAKI, Yimin WANG, Jun KUDOH and Nobuyoshi SHIMIZU (Department of Molecular Biology, Keio University School of Medicine, Tokyo)

Cat eye syndrome (CES) is caused by partial tetrasomy of the 22q11. The morphological anomalies in CES include coloboma of the eye and characteristic heart malformation (totally anomalous pulmonary venous return). Most (>90%) of the CES patient has a supernumerary bisatellited marker chromosome [22pter-q11::22q11-pter] consisting of two copies of the 22pter-q11 region. We have constructed BAC/cosmid contigs of the CES chromosomal region to facilitate isolation of the disease-causing genes.

As DNA resources, we used a CES region-specific cosmid library from the flow-sorted marker chromosomes of a CES cell line (Minoshima et al., this meeting last year) and a BAC library from human total DNA (Asakawa et al., in this meeting). A number of STS's were established from cosmid clones and used to screen CEPH YAC library. Twenty YAC clones were identified and arrayed into a YAC contig. Thus, these YAC clones were used as probes to screen the CES cosmid library and BAC library, and 1,740 cosmids and 98 BAC clones were isolated. To construct BAC/cosmid contigs, the vectorette PCR method and finger printing have been applied. To date, 9 contigs consisting of 25 BAC clones and 24 cosmid clones have been constructed covering over 1.3 Mb. Genomic sequencing of one cosmid clone (36,226 bp) has been completed and other clones are in progress. The genomic sequence information is being used to identify the CES-causing genes.

B-23

A TRANSCRIPT MAP OF THE CANDIDATE GENES IN THE DOWN SYNDROME CRITICAL REGION OF HUMAN CHROMOSOME 21. Nobuyoshi SHIMIZU¹, Nobuaki SHINDOH¹, Hideto MAEDA¹, Akiko YAMAKI², Kazuhiko KAWASAKI¹, Shuichi ASAKAWA¹, Kazutoyo OSOEGAWA³, Eiichi SOEDA³, Yoshiko SHIMIZU², Shinsei MINOSHIMA¹, and Jun KUDOH¹. ¹Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo, ²Dept. Clin. Genet., Sch. Health Sci., Kyorin Univ., Tokyo, and ³RIKEN, Tsukuba Life Sci. Ctr., Tsukuba.

To isolate genes potentially involved in the pathogenesis of Down syndrome, we have performed exon trapping for the "Down syndrome critical region". We have isolated over 100 exons including those from known human genes and homologs of *Drosophila single-minded (sim)* and *minibrain (mnb)* genes. These genes locate in the order of cen-SIM2-HCS-TPRD-MNB-KCNJ6-ERG-tel. *Drosophila sim* and *mnb* genes are required for development of central nervous system. The structural characteristics of human and mouse SIM2 proteins that contain bHLH and PAS domains and expression in the diencephalon during mouse embryogenesis strongly suggest that SIM proteins function as a transcriptional regulator in the development of the central nervous system. Human MNB cDNA encodes a protein kinase with a nuclear targeting signal and a catalytic domain highly homologous to *Drosophila mnb* protein kinase and strikingly resembles the rat Dyrk protein kinase with a dual specificity. The MNB mRNA is expressed in various tissues including fetal and adult brains. These results implicate that human MNB protein may play a significant role in a signaling pathway regulating nuclear functions of neuronal cell proliferation, contributing to certain features of Down syndrome.

B-24

HUMAN BAC LIBRARY: ITS APPLICATION TO MAPPING OF 21q22.3 AND ISOLATION OF DISEASE GENES. Shuichi ASAKAWA, Izumi ABE, Yoshiki KUDOH, Kentaro NAGAMINE, Yimin WANG, Kazuhiko KAWASAKI, Jun KUDOH, Shinsei MINOSHIMA, and Nobuyoshi SHIMIZU (Department of Molecular Biology, KeioUniversity School of Medicine, Tokyo)

We have constructed a human genomic BAC library using a human pre-pro-B cell line. This BAC library consists of 96,000 clones with an average DNA insert size of 110 kb, covering the human genome approximately 3 times. We have established three different screening systems: 1) Probe hybridization to 31 high density replica (HDR) filters: each filter contains 3,072 BAC clones which were gridded in a 6 x 6 pattern, 2) Probe hybridization to 2 Southern blot filters each containing 31 *Hind* III digests of the pooled 3,072 BAC clones. This identifies a particular HDR filter for further probe hybridization, and 3) Two step-polymerase chain reaction (PCR): First PCR is applied to 10 DNA samples from superpools of 9,600 BAC clones to identify a particular superpool and the second PCR applies to 40 DNA samples from 4 dimensionally assigned BAC clones. The two step PCR screening is particularly powerful since it allows us to isolate a desired BAC clone(s) within a day or so. The BAC clones are very useful to construct long range contigs and to utilize starting as a material of sequencing analysis. As a typical example of BAC application, the analysis of 21q22.3 has been presented.

B-25

CHROMOSOMAL LOCALIZATION OF A PSEUDOAUTOSOMAL GROWTH GENE(S). Tsutomu OGATA (1,2), Koji MUROYA (1,2), Yoshimitsu FUKUSHIMA (3), Maki FUKAMI (2,4), Gudrun RAPPOLD (4), Nobutake MATSUO (2) (1, Department of Pediatrics, Tokyo Electric Power Hospital; 2, Department of Pediatrics, Keio University School of Medicine; 3, Department of Hygiene and Medical Genetics, Shinshu University School of Medicine; 4, Department of Human Genetics, Heidelberg University)

The XP/Yp pseudoautosomal region (PAR) carries a pseudoautosomal growth gene(s) (P-growth gene). To localize the P-growth gene(s), we correlated stature with pseudoautosomal genotype in eight Japanese and eight Caucasian non-mosaic patients with partial monosomy of the PAR. The stature was assessed by the comparisons with the race-specific population standards and with the parental heights. The pseudoautosomal genotype was determined for a total of 15 loci by Southern blotting and FISH analysis. Eight patient missing a region between DXYS60–DXYS15 in the distal part of the PAR had definite short stature, whereas four patients preserving the region were normal in height (the remaining four patients were borderline in height and, therefore, excluded from the genotype-phenotype correlations). The results suggest that the P-growth gene(s) is localized to the approximately 350 kb region defined by DXYS60 and by DXYS15. At present, the positional cloning approach is under progress for the P-growth gene(s).

B-26

CHROMOSOMAL LOCALIZATION OF A GENE FOR NON-SPECIFIC X-LINKED MENTAL RETARDATION AT Xp22.3. Maki FUKAMI (1,2), Koji MUROYA (1,3), Nobutake MATSUO (1), Hirofumi OHASHI (4), Yoshimitsu FUKUSHIMA (5), Toshiro NAGAI (6), Gudrun RAPPOLD (2), Tsutomu OGATA (1,3) (1, Department of Pediatrics, Keio University School of Medicine; 2, Department of Human Genetics, Heidelberg University; 3, Department of Pediatrics, Tokyo Electric Power Hospital; 4, Division of Medical Genetics, Saitama Children's Medical Center; 5, Department of Hygiene and Medical Genetics, Shinshu University School of Medicine; 6, Department of Neurology and Medical Genetics, Tokyo Metropolitan Kiyose Children's Hospital)

A gene for non-specific X-linked mental retardation (MRX) has been assigned to the Xp22.3 region. To localize the MRX gene(s), we carried out genotype-phenotype correlations in 13 male patients with partial nullisomy of the X-differential region at Xp22.3. The mental status was assessed by IQ, DQ, and daily life performance. The genotype was determined for a total of 26 loci by PCR and FISH analysis. Three patients missing a region between DXS996 and DXS6834 had obvious mental retardation, whereas the remaining ten patients preserving the region were normal in intelligence. The results suggest that the MRX gene is localized to the approximately 500 kb region between DXS996 and DXS6834. At present, the positional cloning approach is under progress for the MRX gene.

B-27

TURNER SOMATIC STIGMATA: PATHOGENIC ANALYSIS AND CHROMOSOMAL LOCALIZATION OF THE RELEVANT LYMPHOGENIC GENE(S). Koji MUROYA (1,2), Tsutomu OGATA (1,2), Tomonobu HASEGAWA (2), Nobutake MATSUO (2) (1, Department of Pediatrics, Tokyo Electric Power Hospital; 2, Department of Pediatrics, Keio University School of Medicine)

Turner syndrome is associated with characteristic surface and visceral somatic anomalies. Here, we propose that the characteristic Turner stigmata are caused by lymphatic hypoplasia, and attempt to localize the relevant lymphogenic gene(s).

[Lymphatic hypoplasia and Turner somatic stigmata] Turner syndrome is accompanied by lymphatic hypoplasia. Lymphatic hypoplasia causes lymph fluid stasis and resultant distended lymphatics and lymphedema, exerting a mechanical force on the adjacent tissues and organs. Thus, it is hypothesized that Turner somatic stigmata are deformations explained as a malformation sequence initiated by lymphatic hypoplasia. Indeed, Turner somatic anomalies are found in the regions susceptible to the mechanical force initiated by lymphatic hypoplasia.

[Chromosomal localization of the lymphogenic gene(s)]

It has been suggested that the lymphogenic gene(s) for Turner stigmata is shared by Xp and Yp, and haploinsufficiency of the gene(s) results in the development of Turner stigmata. To localize the lymphogenic gene(s) on Yp, we summarized genotype-phenotype correlations in 18 patients with a partial Yp deletion. The results suggest that the lymphogenic gene(s) for Turner somatic stigmata is present in the ZFY-DYS255 region (about 4 Mb in size) in the distal part of Yp.

B-28

GENE EXPRESSION OF A NOVEL LOCUS ON THE X CHROMOSOME WHICH ESCAPES METHYLATION INACTIVATION IDENTIFIED BY TWO-DIMENSIONAL ELECTROPHORESIS OF HUMAN DNA. Jun-ichi ASAKAWA, Mieko KODAIRA, Masaaki IMANAKA, Satomichi KANEOKA, Takahiro TSUJI, Eiko NISHIKORI, Chiyoko SATOH (Dept. Genet. Radiation Effects Research Foundation, Hiroshima)

We have implemented a computer based approach for the analysis of two-dimensional patterns of genomic restriction fragments derived from six mother/father/child trios. We have also undertaken studies of genetic variation which distinguish quantitatively between one- and two-copy fragments. The use of a methylation sensitive cutter for the first dimension separation allows studies of genomic DNA methylation and the identification of novel loci on the X chromosome which escape methylation inactivation. A separate fragment, termed spot 369, was observed as a one-copy spot in male samples, while always as a two-copy spot in female samples. We believe that the *NotI* site of spot 369 on the X chromosome is not methylated on either X in females and thus escapes methylation inactivation. We have cloned a 3.5-kb *NotI/EcoRV* fragment corresponding to this spot. The cloned DNA fragment was mapped to Xp11.4 by FISH analysis. We also cloned and sequenced the 6.5-kb *EcoRV* DNA fragment which contained the *NotI* sites. There was no identical sequence in the DNA data base. Only a few loci have been described on the X chromosome that escape inactivation and have no homologues on the Y chromosome. We have studied the gene expression of this novel locus. We will present preliminary results of a Northern blotting and a cDNA library screening.

B-29

NOVEL POLYMORPHIC MARKER DXYS241 ON THE X-Y HOMOLOGOUS PORTION OF THE HUMAN Y CHROMOSOME AND APPLICATIONS OF ITS POLYMORPHISM. Svetlana E. KOTLIAROVA (Dept. Hum. Genet., Univ. of Tokyo and MEDIGEN Laboratory, Novosibirsk, Russia), Tatsushi TODA, Ikumi MATSUSHITA, Katsushi TOKUNAGA, Yasuo NAKAGOME, Yutaka NAKAHORI (Dept. Hum. Genet., School of Int.Health, Univ. of Tokyo)

Y chromosome is unique because it represents the patrilineal contribution to the male genome. X-Y homologous non-recombining portion of Y chromosome is especially interesting in respect that it can provide information both on the male-specific gene flow and on the sex chromosome evolution studies. However, the number of polymorphic markers developed on this portion of Y chromosome is still scarce. Hereby we present a new polymorphic (CA)_n microsatellite marker DXYS241 developed on the non-recombining portion of X-Y homologous region of Yp11. The alleles at this locus are found to be associated with sex chromosomes. Three differences were detected between Y and X chromosome-specific alleles, allowing sex-chromosome-specific allele amplification. The marker was polymorphic in three examined ethnic groups: Japanese, US Caucasians and US Negroids. A total of 13 different alleles and 27 genotypes are detected in the studied 136 individuals. Among the Y-chromosome specific alleles population-associated alleles are revealed. DXYS241 can be applied for a variety of purposes: for tracing male-specific background; for haplotype construction on the human Y chromosome and for sex determination. It is potentially useful informative marker for forensics and for other genetic studies.

B-30

ANALYSIS OF THE X-Y HOMOLOGOUS REGION SURROUNDING THE AMELOGENIN GENE. Ayuchi TSUBOI, Bunkichi TSUNEKAWA, Akiko HIDA, Ikumi MATSUSHITA, Tatsushi TODA, Katsushi TOKUNAGA and Yutaka NAKAHORI (Dept. Hum. Genet., Univ. of Tokyo, Tokyo)

It has been proposed that the human sex chromosomes evolved from a pair of homologous autosomes which eventually became different in structure from each other. However, there are a number of regions on these sex chromosomes that retain homology, and genes that were conserved on the Y chromosome may exist in these X-Y homologous regions.

The amelogenin gene is located in one of the X-Y homologous regions; Xp22.2 and Yp11.2. Since several disease genes are located in the Xp22.2-22.3 region, we are aiming to clone novel genes from the region surrounding the amelogenin gene.

The 540kb Y-derived YAC (CEPH 235F3) that contains the amelogenin gene has 2 CpG islands, and by utilizing them as markers for screening the cDNA library, several clones were obtained. Sequence analysis of these clones showed that a clone from one of the CpG islands is highly homologous to the endogenous retrovirus, and the clone from the other CpG island contained a 1kb open reading frame. The latter clone may be a novel gene, and further analysis, including the existence of a homologue on the X chromosome, is under way.

B-31

A POLYMORPHISM IN THE EXON OF THE SRY GENE AND HAPLOTYPING OF THE Y CHROMOSOME IN JAPANESE. Keiko TOMITA, Tatsushi TODA, Aoi JO, Katsushi TOKUNAGA, Yasuo NAKAGOME, Yutaka NAKAHORI (Dept. Hum.Genet.,Univ. of Tokyo, Tokyo) and Toshikatsu SHINKA (Dept.Pediat., Tohoku Univ.,Sendai)

We described a new substitution polymorphism in the coding sequence of the testis determining gene SRY. The frequency of the new allele in Japanese was 30%, which was almost the same as in Korean, whereas it was not observed among Caucasians and Negroes. The combination analysis with the other two polymorphic loci on the Y chromosome indicated that the base substitution occurred before the rise of Y2 allele of DXYS5Y and after the branching of the YAP(+) and YAP(-) alleles of DYS287. Since the Y2 allele is geographically restricted to the narrow regions of east Asia, the SRY polymorphism may contribute to the exploration of the origin of Japanese.

B-32

ANALYSIS OF DELETED REGION ON Y CHROMOSOME LONG ARM IN AZOOSPERMIC PATIENTS. Yoko KUROKI, Rie KOMAKI, Kazuhiro KOBAYASHI, Jing ZHANG, Tatsushi TODA, Yasuo NAKAGOME, Katsushi TOKUNAGA, Yutaka NAKAHORI (Dept. Hum. Genet., Univ. of Tokyo, Tokyo) Mikio NAMIKI (Dept. Urol., Kanazawa Univ., Ishikawa) Teruaki IWAMOTO (Dept. Urol., St. Marianna Univ., Kanagawa)

We have previously reported the results of patient analysis with either azoospermia or oligozoospermia whose Y chromosomes were cytogenetically normal. The results by Southern hybridization and PCR showed a common interstitial micro-deletion involving DYS7C and DYS1 loci on the long arm of the Y (Yq) in almost one tenth of the patients. Recently, two candidate genes for azoospermia have been cloned from the azoospermic factor (AZF) region on Yq, but a point mutation of these genes has not been detected in patients. Interestingly, although these patients seem to possess common micro-deletion in the same region, they exhibit different phenotypes. Therefore we assume that the region around the breakpoint is critically important for spermatogenesis. We made the restriction map of a YAC clone (yOX21) spanning the proximal breakpoint, and as a result, we recognized the distance between DYS7C and the breakpoint to be not more than 260Kb. Using the cosmid library prepared from the YAC, we are currently constructing the cosmid contig covering the proximal breakpoint.

B-33

DETECTION OF SEX SPECIFICALLY EXPRESSED GENES USING DIFFERENTIAL DISPLAY. Sumiyo MORITA, Tatsushi TODA, Katsushi TOKUNAGA, and Yutaka NAKAHORI (Dept. Hum. Genet., Univ. of Tokyo, Tokyo) and Taku IIDA (Dept. Obstet. and Gynecol., Univ. of Tokyo, Tokyo)

In human, development into males is determined by the presence of the Y chromosome. The testis determining factor SRY was cloned in 1990. After that, several genes have been proposed as factors for sex determination or sex differentiation, although the function and the relationship between each factor are yet unknown. Recently it has been supposed that the different species have their own mechanism of sex determination, and even in mammals the scheme may not be completely the same. The sex determination of human is thought to occur at the 7 weeks of gestation. To elucidate the sex determining path in human, we used the Differential Display technique to detect the differential transcripts between male and female in the crucial period. Currently, several mRNAs are supposed to be differently expressed and the clones are investigated.

B-34

Developmental genetic analysis of mouse neurological mutant, *quaking*;
Identification of responsible gene, *qkI*.

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Mouse *quaking* (*qk*) mutation is a pleiotropic locus required for myelination, embryogenesis, and spermiogenesis. After detailed structural characterization of the *qk* genomic region, a strong candidate gene for *qk* mutation has been found adjacent to the deletion break point in the *qk^v* mutant genome, and designated as *qkI*. This gene encodes a putative RNA binding protein, and show predominant expression in myelinating brain and in embryos. However, *qkI* gene itself is not structurally altered in the mutant genome, and also the level of expression is not changed from normal mice. In order to formally prove that *qkI* is the responsible gene for *qk* mutation, we have produced *qkI* gene knockout mice. It was found that double heterozygote mice for *qk^v* and this *qkI*-KO allele indeed developed tremor, unambiguously demonstrating that the association of the *qkI* gene with the *quaking* neurological phenotype.

B-35

FUNCTIONAL ANALYSIS OF MOUSE NEUROLOGICAL MUTANT, *quaking*.

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Mouse *quaking* (*qk*) mutation is a pleiotropic locus required for myelination, embryogenesis, and spermiogenesis. The mouse *quaking* is thought as a model of one type of dysmyelinating disorder. Recently, a strong candidate gene for *qk* mutation has been found adjacent to the deletion break point, and designated as *qkI* (Ebersole *et al. Nature Genetics* 12, 260-265, 1996).

This *qkI* gene is expressed three types of mRNA caused by alternative splicing. The gene encodes a putative RNA binding protein with homology to human Src-associated protein Sam 68. To know function of its product, we have produced *qkI* gene knockout mice by inserting IRES-βgeo into a highly conserved domain of the gene. It was found that compound heterozygote mice for *qk^v* and this *qkI*-KO allele indeed developed tremor, directly demonstrating the association of the *qkI* gene with the *quaking* phenotype. Besides, the gene was expressed neural tissues and heart during embryogenesis from 8.5 day of pregnancy and in adult. *qkI* gene was also expressed during myelin formation in the brain.

Thus, we concluded that the *qkI* gene product plays an important role during myelination.

B-36

LINKAGE MAPPING OF CHARCOT-MARIE-TOOTH DISEASE TYPE 2A (CMT2A) TO CHROMOSOME 1p. Masaaki. SAITO¹, Yasuko. HAYASHI¹, Takashi. SUZUKI², Hajime. TANAKA¹ and Shoji. TSUJI¹. (1; Dept. Neurol. Brain Research Inst., Niigata Univ., Niigata. 2; Dept. Neurol., Toyama Central Hospital, Toyama.)

Charcot-Marie-Tooth disease type 2 (CMT2) is a genetically heterogeneous disorder with at least two loci identified; 4 families with CMT2A (1p35-36) and 1 family with CMT2B (3q13-22). By the linkage analysis of 2 Japanese CMT2 families (family 693 and family 694), the highest multipoint lod scores of 2.01 at D1S244 and 1.93 at D1S228 were obtained for family 694 and family 693, respectively, and the highest cumulative multipoint lod score of 3.69 was obtained at D1S244. Obligate recombination events at D1S160 and D1S228 as well as the multipoint linkage analysis indicate that the gene for CMT2A is likely to be localized within a 14.7 cM region as a 95% confidence interval. Clinical characteristics of the two CMT2A families include the following; 1) the posterior tibial muscles are often as severely involved as the anterior tibial muscles, 2) generalized areflexia is infrequent, 3) peripheral nerves are not clinically thickened and 4) sensory loss is infrequent.

B-37

MUTATIONS IN THE *TRKA* /HIGH AFFINITY NGF RECEPTOR GENE IN PATIENTS WITH CONGENITAL INSENSITIVITY TO PAIN WITH ANHIDROSIS. Yasuhiro INDO, Motoko TSURUTA, Yumi HAYASHIDA, Md Azharul KARIM, Kohji OHTA, Tomoyasu KAWANO, Hiroshi MITSUBUCHI, Ichiro MATSUDA (Dept. Pediat., Kumamoto Univ., Kumamoto), Hidefumi TONOKI (Dept. Pediat., Hokkaido Univ., Sapporo), and Yutaka AWAYA (Dept. Pediat., Seibo Hospital, Tokyo)

Congenital insensitivity to pain with anhidrosis (CIPA) is an autosomal-recessive disorder characterized by recurrent episodes of unexplained fever, anhidrosis (absence of sweating) and absence of reaction to noxious stimuli, self-mutilating behavior, and mental retardation. The genetic basis for CIPA is unknown. Nerve growth factor (NGF) induces neurite outgrowth and promotes survival of embryonic sensory and sympathetic neurons. Mice lacking the gene for TrkA, a receptor tyrosine kinase for NGF, share dramatic phenotypic features of CIPA, including loss of responses to painful stimuli, although anhidrosis is not apparent in these animals. We therefore consider the human *TRKA* homologue as a candidate for the CIPA gene. The mRNA and genomic DNA encoding for TRKA were analyzed in three unrelated CIPA patients who had consanguineous parents. We detected a deletion-, splice-, and a missense-mutation in the tyrosine kinase domain in these three patients. Our findings strongly suggest that defects in *TRKA* cause CIPA and that the NGF-TrkA system has a crucial role in the development and function of the nociceptive reception as well as establishment of thermoregulation via sweating in humans. These results also implicate genes encoding other TRK and neurotrophin family members as candidates for developmental defect(s) of nervous system.

B-38

A de novo insertional mutation of the P0 gene in a patient with congenital hypomyelination neuropathy. Naoki KOSUKA, Nobutada Tachi (Sch. of Health Sci., Sapporo Med. Univ., Sapporo), Kazuhiro OHYA, Shunzo CHIBA (Dept. of Pediatr., Sapporo Med. Univ., Sapporo), and Sumimasa YAMASHITA (Kanagawa Children's Med. Center of Yokohama, Kanagawa)

We present a patient that corresponds to a diagnostic criteria of congenital hypomyelination neuropathy (CMT type 3) and disclosed a de novo insertional mutation of the P0 gene. This case was 8-year-old-boy. He had been normal pregnancy and delivery. His motor function and milestones were retarded by muscle hypotonia. At 4 year, he could walk with support. Physical examination revealed the patient to be well nourished and in a normal mental state, but he had mild hypotonia with predominantly distal muscle weakness. He showed Gower's sign. There were no signs of cerebellar dysfunction. In gene analysis, the patient showed no CMT1A duplication within the chromosome 17p11.2-12 by Southern blot hybridization of MspI digested genomic DNA with probe pVAW409R3. By heteroduplex analysis of exon 4 in P0 gene, a heteroduplex band was detected in the patient but not in his parents. Direct nucleotide sequence determination was identified a GGCA insertion at position between nucleotide 560 and 561. This mutation was not identified in his parents. This is a first case of congenital hypomyelination neuropathy who had a de novo insertional mutation of the P0 gene.

B-39

CLINICAL AND MOLECULAR GENETIC STUDY OF JUVENILE MYOCLONIC EPILEPSY. Miyako OGUNI, Hirokazu OGUNI, Kayoko SAITO, Makiko OSAWA (Dept. Pediatr, Tokyo Women's Medical College, Tokyo), Naoyuki KAMATANI (Dept. Rheumatology), Yukio FUKUYAMA (Pediatric neurological institute), Tetsuro MIKI, Koujin KAMINO (Dept. Internal Med, Osaka university, Osaka)

Juvenile myoclonic epilepsy (JME) is the most well established syndrome among the idiopathic epilepsies. JME aggregates in families, but the exact mode of inheritance remains unknown. Segregation analysis has been undertaken since Greenberg et al. first reported an evidence favoring linkage of a locus predisposing to JME to the HLA region of chromosome 6p. Subsequently, Liu et al. showed that an epilepsy locus existed on chromosome 6p21.2-p11. We conducted pairwise linkage analysis of JME families as a means of assessing autosomal recessive inheritance.

The diagnosis was confirmed by the clinical and EEG findings in 8 families. Family members were typed for 11 polymorphic loci on chromosome 6p. The maximum lod score in this region was 3.3. Further investigation with a larger number of families is planned to confirm whether chromosome 6p21.2-p11 is a genetic locus of JME.

B-40

Induction of NAIP mRNA in the brain produced by middle cerebral artery occlusion in rat.

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<Introduction> Neuronal apoptosis inhibitory protein (NAIP) presumed to have a strong relations to neuronal cell death in various conditions. The purpose of our study is to evaluate the expression of NAIP mRNA in rat focal brain ischemia model (middle cerebral artery model, MCA occlusion), topologically and chronologically. **<Materials and Methods>** Eight SD rats were anesthetized by 2% halothane and MCA occlusion was made by Tamura's method. The brain was removed 0, 1, 3 and 24 hours after occlusion and frozen at -40°C. Brains were stored at -80°C. Ten µm thick coronal brain sections were cut by cryo-microtome through the MCA territory. Digoxigenin(DIG)-labeled RNA probe was designed by targeting NAIP mRNA sequences including BIR domain conserved in other IAP(inhibitor of apoptosis) families. Hybrid RNA probe was detected by using DIG-label in-situ hybridization method. NAIP was also stained by immunohistochemical method. **<Results>** Fine signals were observed symmetrically in cortex and striatum in normal brain. Clear signals were seen 1 hour after occlusion in infarct and penumbra-area (adjacent to the infarct area). Three hours later occlusion, the intensity of signals were increased and extended in injured hemisphere. Although signals were disappeared in the core of infarction, The signal was apparently demonstrated in penumbra-area and a part of contralateral hemisphere. The development of NAIP was not clearly correlate with that of NAIP mRNA. **<Conclusion>** NAIP mRNA was induced by ischemic stress in rat brain. NAIP presumed to be necessary for insulted neuronal cells to survive, because the expression of NAIP mRNA was prominent in penumbra.

B-41

DIFFERENT ORIGINS OF EXPANDED CAG REPEATS IN DENTATORUBRAL AND PALLIDOLUYSIAN ATROPHY AND HAW RIVER SYNDROME. Hiroko YANAGISAWA^{1,2}, Shigeo NAGAFUCHI¹, Yutaka NAKAHORI² & Masao YAMADA¹. (¹ Natl. Children's Med. Res. Ctr., 3-35-31, Taishido, Setagaya, Tokyo 154, ² Dept. of Human Genetics, Univ. of Tokyo)

Dentatorubral and pallidolusian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder, and has been shown to be due to an expanded trinucleotide repeats in a coding region of the DRPLA gene located on 12p13.31. The prevalence of DRPLA is quite low in Europeans compared to the Japanese population. Outside Japan, however, 8 DRPLA kindreds were so far detected by molecular analysis. Haw River Syndrome (HRS) which occurs in a large African-American family residing in rural areas of North Carolina has also shown to be caused by expansion of the same repeat as DRPLA. Although the two disorders show similar clinical observations, some differences are noted including the absence of genetic anticipation in HRS and the presence of extensive subcortical white matter demyelination, basal ganglia calcifications and neuroaxonal dystrophy in HRS. We have been analyzing haplotypes around the DRPLA gene and repeats number in a various of DNA samples. All the expanded repeats of DRPLA patients in Japan and of two Caucasian DRPLA kindreds were exclusively associated with the A1-B1 haplotype, suggesting a unique origin for expanded DRPLA repeats (Yanagisawa et al. Hum. Mol. Genet. 5, 373-379, 1996). We have now analyzed three additional Caucasian DRPLA kindreds, which confirms the previous observation. In contrast, the HRS patients have expanded repeats on chromosomes with a different haplotype (A1-B2). This indicates a second predisposing haplotype in Africans as in the case of myotonic dystrophy.

B-42

MOLECULAR GENETIC DIAGNOSIS OF SPINAL MUSCULAR ATROPHY. Yumi SHIRAIWA, Kayoko SAITO, Haruko SUZUKI, Keiko SHISHIKURA, Makiko OSAWA, Yukio FUKUYAMA (Dept. Pediatri., Tokyo Women's Medical College, Tokyo), Jo-EIKEDA (Institute of Molecular Science, Tokai University, Kanagawa), Hiroyosdi KOIDE (Dept. Pediatri., Saitama Medical College, Saitama), Toshiyuki KUMAGAI (Dept. Pediatri., Aichi Prefectural Colony Central Hospital, Aichi).

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by primary degeneration of the anterior horn cells of spinal cord. We examined two candidate genes, NAIP (neuronal apoptosis inhibitory protein) gene and SMN (survival motor neuron) gene. The subjects included <group A> 38 SMA patients (12 cases of type I, 19 of type II and 7 of type III), <group B> 53 normal individuals from families with SMA patients and <group C> 26 normal controls. Exon 7 and 8 of the SMN gene and exon 4, 5 and 6 of the NAIP gene were amplified by PCR. Thirty-three SMA cases (including 10 of type I, 17 of type II and 3 of type III) showed deletions of SMN telomeric copies of both exon 7 and 8 (0/group B, 0/group C). One case of each type showed the deletion of only SMN exon 7. In another 5 cases (i.e., 1 case of type I, 1 of type II and 3 of type III), no deletion was found of either exon 7 or 8. Two cases of SMA type I also showed the deletion of exon 5 of the NAIP gene (0/group B, 0/group C) as well as exons 7 and 8 of the SMN gene. In the other 36 SMA patients, no deletion was detected in exon 5 of the NAIP gene.

B-43

CLONING OF THE TWO GENES RESPONSIBLE FOR AN AUTOSOMAL RECESSIVE MUSCULAR DYSTROPHY, SCARMD. Mikiharu YOSHIDA, Satoru NOGUCHI, Yuji MIZUNO, Yasuko HAGIWARA, Ikuya Nonaka and Eijiro OZAWA (National Institute of Neuroscience, NCNP, Tokyo)

SCARMD is a Duchenne-like but an autosomal recessive muscular dystrophy. As a protein responsible for SCARMD, adhalin, one of dystrophin-associated proteins (DAPs), was focused in 1992. Soon, however, SCARMD was shown to be genetically heterogeneous diseases. In 1994 on the basis of our results, we proposed a hypothesis that SCARMD is the disease deficient in the sarcoglycan (SG) complex rather than adhalin. The complex, which we biochemically found, is composed of three DAPs called α -, β - and γ -SGs and adhalin corresponds to α -SG. The heterogeneity of SCARMD can be explained on the assumption that, if any one of the SG genes is defective, the SG complex would not be formed on the sarcolemma and function any more.

In this study, we have cloned the cDNAs of β - and γ -SGs and determined their gene loci on the human chromosomes. We have identified the SCARMD patients with defect in either of the SG genes and found in each case that not only the corresponding SG but also the other SGs whose genes are normal were lost in the skeletal muscle. These findings strongly support our hypothesis. We propose the name sarcoglycanopathy for SCARMD. We assume that the SG complex, whose function is yet unknown, must play an important role to keep muscles from degeneration.

B-44

MOLECULAR GENETIC EVIDENCE OF CLINICAL HETEROGENEITY IN FUKUYAMA TYPE CONGENITAL MUSCULAR DYSTROPHY Eri KONDO-IIDA, Kayoko SAITO, Makiko OSAWA (Dept. Pediatr., Tokyo Women's Medical College, Tokyo), Hajime TANAKA, Shoji TSUJI (Dept. Neurol., Brain Reserach Institute, Univ., Niigata, Niigata), Tadayuki ISHIHARA (Div.Internal Med., National Higashisaitama Hosp.,Saitama) and Tatsushi Toda (Dept. Human Genet., Univ. Tokyo, Tokyo)

A small fraction of Fukuyama type congenital muscular dystrophy (FCMD) patients acquire the capacity to walk. Whether such ambulant cases belong to the classical FCMD spectrum or a different entity has been a topic of considerable debate. We performed linkage analysis for 10 families with ambulant cases, using DNA markers flanking the FCMD locus. The mfd220 locus yielded a significant lod score of 3.09 for ambulant FCMD. We also found evidence for linkage disequilibrium between ambulant FCMD and mfd220. We also conducted haplotype analysis in two FCMD siblings with different phenotypes, one of whom was ambulant while the other was not. The results indicate that the two sets of FCMD siblings share exactly the same haplotype surrounding the FCMD locus. On the basis of these results, we conclude that, genetically, ambulat cases are, in fact, part of the FCMD spectrum.

B-45

PRENATAL DIAGNOSIS OF FUKUYAMA TYPE CONGENITAL MUSCULAR DYSTROPHY USING POLY-MORPHISM ANALYSIS. Kayoko SAITO, Eri KONDO, Kiyoko IKEYA, Yumi SHIRAIWA, Makiko OSAWA, Yukio FUKUYAMA (Dept. Pediatr., Tokyo Women's Medical College, Tokyo), Tatsushi TODA (Div. Hum. Genet.,Tokyo University, Tokyo) and Tomoko YAMAMOTO, Makio KOBAYASHI (Dept. Pathol.,Tokyo Women's Medical College,Tokyo)

We reported the first successful prenatal diagnosis in two Fukuyama type congenital muscular dystrophy (FCMD) families. We expanded this topic in 6 additional families. Three affected fetuses were examined neuropathologically. Ethical issues were carefully considered in each case. DNA was extracted from chorionic villi or amniocytes sampled at 10 to 17 gestational weeks (GW). The haplotypes of the family members and fetuses were analysed with polymorphic microsatellite markers flanking the 9q31-33 locus. The parents were informed of the results at 12 to 19 GW. The fetuses in families 1,4,6,7 and 8 were diagnosed as being unaffected. The babies in families 1 and 4 are healthy. In families 6,7 and 8, babies are due in November, January and February, respectively. The fetuses in families 2,3 and 5 were revealed to be affected, and the parents opted for abortion. The family 2 fetal brain(20GW) macroscopically showed small multiple granular protrusions over the cerebral surface composed of aberrant neuroglial clusters, as judged on microscopy, which migrated out through the discontinuous pial-glial barrier into the extra-cortical glial layer. The family 3 and 5 fetal brains (18 and 17GW, respectively) showed the same findings, as the initial stage of cortical dysplasia, though milder.

B-46

HAPLOTYPE MAPPING OF THE FUKUYAMA-TYPE CONGENITAL MUSCULAR DYSTROPHY (FCMD) GENE.

Kazuhiro KOBAYASHI, Masashi MIYAKE, Katsushi TOKUNAGA, Yutaka NAKAHORI and Tatsushi TODA. (Dept. Hum. Genet., Univ. Tokyo, Tokyo.)

Fukuyama-type congenital muscular dystrophy (FCMD) is an autosomal recessive severe muscular dystrophy associated with brain malformation. After our initial mapping of the FCMD locus to 9q31-33, we have found linkage disequilibrium between FCMD and D9S306 on 9q31, performed linkage-disequilibrium mapping using new microsatellites near D9S306, and presumed that the FCMD gene lies within a region of <100kb containing D9S2107. In the present study, we developed two new microsatellites (E6 and J7) between D9S2105 and D9S2107, determined their location, and examined haplotypes of FCMD chromosomes using the four markers (cen-D9S2105-E6-J7-D9S2107-tel). Eighty % of FCMD chromosomes shared the founder haplotype (138-6-8-8) and 95% of FCMD patients carried either two founder haplotypes homozygously or one founder haplotype and one putative newly mutated chromosome heterozygously. This haplotype has not been observed in normal chromosomes yet. These findings verify our hypothesis of a single disease founder in the Japanese population. Only several kinds of haplotype different from the founder's one were observed in FCMD chromosomes, indicating that mutation events in the FCMD gene have happened so far at most several times except the founder mutation event. Moreover, we found several historical recombinations disrupting the founder haplotype at D9S2105 or E6, suggesting that the FCMD gene is likely to be located between D9S2105 and E6. Taken together with our previous study, the FCMD gene lies just proximal to the marker E6.

B-47

POINT MUTATION OF THE CARDIAC β -MYOSIN HEAVY CHAIN GENE WITHOUT AMINO ACID EXCHANGE IN FAMILIAL HYPERTROPHIC CARDIOMYOPATHY.

Kiyomi NIKI, Michiko FURUTANI, Shouichi ARAI, Shin-ichiro IMAMURA, Kazuo MOMMA, Saichi HOSODA and Rumiko MATSUOKA

(The Heart Institute of Japan, Tokyo Women's Medical College)

Point mutation of the cardiac β -myosin heavy chain (β -MHC) gene without amino acid exchange (silent mutation) has been detected in familial hypertrophic cardiomyopathy patients (FHC). We investigated the relation between this silent mutation and the clinical signs of hypertrophic cardiomyopathy (HCM) in a FHC. One family of FHC (5 HCM patients and 11 unaffected family members), and 50 normal subjects were examined for the point mutation of β -MHC gene. Point mutation of β -MHC without amino acid exchange in exon 3 was found in all HCM patients (Thr 63 Thr). The same mutation was detected in normal subjects but at a much lower rate than HCM patients (20% vs 100%). This mutation was also detected in 5 of 11 unaffected family members (45%); 4 of the 5 are under 30 years old and require further follow up. The high rate of the same silent mutation found in familial hypertrophic cardiomyopathy patients suggests that silent mutation may be associated with hypertrophic cardiomyopathy.

B-48

STRUCTURAL ANALYSIS OF *Ampd3* GENE ENCODING HEART-TYPE AMP DEAMINASE. Takayuki MORISAKI, Kannika SERMSUVITAYAWONG, Xudong WANG, Keiko TOYAMA, Hiroko MORISAKI, Tsunehiro MUKAI (Dept. Bioscience, Natl. Cardiovasc. Ctr. Res. Inst., Osaka), Ikuo MINEO (Otemae Hosp., Osaka) and Nobuaki OGASAWARA (Aichi Pref. Colony, Aichi)

Myoadenylate deaminase (muscle AMPD) deficiency is one of the most common inherited enzyme defect. Although a point mutation (34C to T) in *AMPD1* gene is thought to be responsible for all muscle AMPD deficiency examined thus far, many individuals with this mutation show no clinical symptom. This could be explained by gene regulatory mechanisms including alternative splicing and expression of other *AMPD* genes. To investigate these mechanisms in animal models, we isolated mouse *Ampd3* gene encoding the heart-type AMPD, which is also expressed in skeletal muscles. Prokaryotic expression of the isolated gene confirmed that *Ampd3* encodes the heart-type isozyme. Mouse *Ampd3* gene was found to be more than 40 kb in length, located on mouse chromosome 7 and consist of 15 exons. Although mouse *Ampd3* was found to be quite homologous to human *AMPD3*, the expression pattern and the structure of the 5' region of mouse *Ampd3* were distinct from those of human *AMPD3*. These observation will give us a clue to understand the function of *Ampd* gene family in animal models.

B-49

MUTATION IN AT LEAST 7 DEFFERENT CONTRACTILE ELEMENTS CAUSES HYPERTROPHIC CARDIOMYOPATHY. Akinori KIMURA, Haruhito HARADA, Shitoshi HIROI, Shu-Reng DUAN, Manatsu SATO, Megumi TAKAHASHI, Taishi SASAOKA, Takeyuki NAKAMURA, and Nobuhisa OHBUCHI (Dept. Tissue Physiol., Div. Adult Dis., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterized by cardiac hypertrophy accompanied by myofibrillar disarrays. Genetic linkage studies have revealed at least 4 disease genes; cardiac β myosin heavy chain (c β MHC, *CMH1* locus, 14q2), cardiac troponin T (cTnT, *CMH2* locus, 1q3), α tropomyosin (α TM, *CMH3* locus, 15q1), and cardiac myosin binding protein C (cMBPC, *CMH4* locus, 11p1) genes. To decipher the molecular basis of HCM in Japan, we have searched for mutations in these HCM genes in a large panel (n=135) of Japanese patients. Mutations in the c β MHC, cTnT, α TM, and cMBPC genes were identified in 23%, 15%, 1%, and 10% of the patients, respectively, i.e. about 49% of Japanese HCM was attributable to mutations in the known HCM genes. Because these known HCM genes encode for major contractile elements in the cardiac muscles, we have searched for mutations in the other contractile element genes. By this candidate gene approach, we found mutations in ventricular myosin essential light chain, ventricular myosin regulatory light chain, and cardiac troponin I genes in a few patients. These studies have shown that there are at least 7 different HCM genes accounting for about 53% of HCM in Japan.

B-50

MORTALITY OF WILSON DISEASE IN JAPAN, 1969-1994.
Yoko Imaizumi (Institute of Population Problems, Ministry of Health and Welfare, Tokyo)

The death rate of Wilson disease (WD) was analyzed using Japanese vital statistics for the period 1969-1994. Data consisting of 503 (289 males and 214 females) deaths from WD were obtained during that period. The age-adjusted death rates of WD per million population were 0.57 for males and 0.36 for females in 1969 and decreased significantly year by year. The death rate was significantly higher in rural (0.20) than in urban (0.16) areas during that period. There were remarkable differences in the WD death rates among the four marital categories. The lowest age-adjusted death rate was seen in the married group for males (0.08) and females (0.07), whereas the highest rate was seen in the widowed group for males (1.02) and in the single group for females (0.45). The mean age at death from WD was 38 years for both sexes in 1969-1972 and decreased with the year, where the overall mean age at death was about 33 years for both sexes.

B-51

MOLECULAR CHARACTERIZATION OF GALACTOSEMIA MUTATIONS (TYPE 1) IN JAPANESE. Hidetetsu HIROKAWA, Yosiyuki OKANO, Takuji IMAMURA, Gen ISSHIKI (Dept. Pediatr. Osaka City University Medical School) and Itsujin SUYAMA (Osaka Municipal Rehabilitation Center for the Disabled)

Galactosemia (type 1) is an autosomal recessive genetic disorder due to galactose-1-phosphate uridylyltransferase (GALT) deficiency. Recently we characterized 7 novel mutations of GALT gene in 11 patients from 9 families. All patients' GALT activities in the erythrocytes were reduced. The 7 novel mutations were 5 missense mutations (M142V, G179D, W249R, P325L, R333Q), a nonsense mutation (W249X), and a deletion (977C-del). These 7 mutations have not been found in Caucasians yet. Therefore, we suggest the mutation due to galactosemia occurred after racial divergence between Caucasians and Asians. We recreated these mutant GALT cDNA by site-directed mutagenesis. The mutant constructs have been transfected into COS cells. We analyzed GALT activities in COS cells. The results showed all mutants' GALT activities were reduced and these missense mutations were the cause of GALT deficiency. The values significantly related to the GALT activities in the erythrocytes of the patients.

B-52

cDNA ANALYSIS OF A MALE CASE WITH GALACTOKINASE DEFICIENCY. Akemi HAZEYAMA, Hiroshi NAKANO, Hitoshi ISHIZAKA, Hiroko HARASHIMA, Akira OHTAKE, Nozomu SASAKI (Dept. Pediatr., Saitama Medical School, Moroyama, Saitama), Akie FUJIMOTO (Osaka City Environment and Public Health Association, Osaka) and Yoshiyuki OKANO (Dept. Pediatr., Osaka City Univ. Medical School, Osaka)

We describe here a cDNA analysis of a Japanese male case with galactokinase deficiency. He was the first child of healthy and nonconsanguineous parents. The blood galactose concentration was detected to be high on newborn screening. The galactokinase activity of the proband using red blood cells was 6.6% of controls and those of parents are 20% and 47%, respectively. Dietary treatment was started immediately. Since then he does not have cataracts and is developing normally at the age of 1 year and 8 months. We used the reverse transcription-polymerase chain reaction to obtain cDNAs encoding galactokinase from lymphoblastoid cell lines. G1045A(Gly349Ser), was identified homozygously in the proband and heterozygously in his mother. Gly349 takes part in the formation of ATP-binding motif and conserves among all the galactokinases.

B-53

ACCUMULATION OF GM2 GANGLIOSIDE IN NIEMANN-PICK DISEASE TYPE C. Tamami YANO, Miyako TANIGUCHI, Kousaku OHNO (Dept. Neurobiology, School of Life Science, Tottori Univ.), Marie T. VANIER (Dept. Biochemistry, INSERM-CNRS, Lyon-Sud School of Medicine, Oullins Cedex, France), and Goro TAKADA (Dept. Pediatrics, School of Medicine, Akita Univ.)

We have found that Niemann-Pick disease type C (NP-C) fibroblasts have an increased level of bound sialic acid. To characterize the bound sialic acid, we have studied intracellular ganglioside. GM2 ganglioside (GM2) was accumulated in most of NP-C cells. The incorporations of [³H] mannosamine into GM2 and GM3 ganglioside fractions were also increased. Cells cultured in lipoprotein-depleted medium diminished the accumulation of cholesterol but not of GM2. It is unlikely that the GM2 accumulation arises from a secondary lysosomal function caused by the storage of excess cholesterol. It is suggested that the accumulation of GM2 together with cholesterol is a unique abnormality in NP-C cells and the defect in NP-C may be involved in intracellular transport of both cholesterol and GM2.

B-54

PRENATAL EXCLUSION OF CARBAMYLPHOSPHATE SYNTHETASE-I DEFICIENCY BY FETAL LIVER BIOPSY.

Makoto YOSHINO, Atsushi NISHIYORI, Yasutoshi KOGA, Hirohisa KATO (Dept. Pediatr. & Child Health), Shigeki IZUMI, Tetsuro HATASE, Michiaki YAKUSHIJI (Dept. Obst. Gynecol., Kurume Univ. Sch. Med.)

A prenatal enzymatic examination for carbamylphosphate synthetase-I (CPS-I) deficiency was carried out in a pregnant woman at risk for the disease at 21 weeks of gestation. Fetal liver tissue was obtained by aspiration needle biopsy. The activity of CPS-I in the fetus at risk was 4.9% of mean values of fetal tissue at 18 to 21 weeks of gestation. The activity of ornithine transcarbamylase (OTC) was also low at 8.9% of the control mean value. We interpreted these apparent low values were due to contaminating blood clotting, and decided that the fetus was unaffected because (1) the fetal CPS-I activity was within detectability, in contrast to the complete absence of CPS-I in the propositus' liver tissue, and because (2) the activity ratio of CPS-I to OTC was 55% of the control mean value. The pregnancy was continued and the baby was delivered at 40 weeks of gestation and confirmed to be unaffected. Comparison with the propositus' CPS-I activity and the activity ratio may be useful to differentiate whether or not the fetus is affected when a low activity is elicited.

B-55

MOLECULAR CHARACTERIZATION OF PERSISTENT HYPERMETHIONINEMIA WITH DOMINANT INHERITANCE.

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We describe a type of mild hypermethioninemia due to a point mutation in the hepatic MAT gene, which was inherited in a dominant fashion in a family. Three patients coming from a same family pedigree were ascertained by the presence of isolated hypermethioninemia on mass-screening program. The measurement of MAT activity in a patient's liver indicated a mild partial deficiency of the high Km form of hepatic MAT. SSCP analysis and direct sequencing of patient's genomic DNAs revealed a G to A mutation at nucleotide 791 that converts Arg-264 to His (R264H) in one allele of MATA1 gene. Gene tracking in the family revealed that the hypermethioninemia is associated with heterozygosity for the R264H mutation in the MATA1 gene. (R264H mutation; J.Y.Chou et al. 1996)

B-56

IDENTIFICATION OF MISSENSE MUTATIONS AND HAPLOTYPING OF CARNITINE PALMITOYLTRANSFERASE II GENE.

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Carnitine palmitoyltransferase II (CPTII) deficiency manifests as various clinical phenotypes: a relatively mild form with muscular symptoms and a severe phenotype presenting with hepatocardiomyopathy. We have investigated three Japanese patients with CPT II deficiency. Molecular analysis revealed two novel missense mutations, a glutamate (174)-to-lysine substitution (E174K) and a phenylalanine (383)-to-tyrosine substitution (F383Y) in the CPTII cDNA. Transfection experiments demonstrated that the two mutations reduced CPTII catalytic activity. Case 1 (muscular form) was homozygous for the E174K mutation, whereas case 2 (hepatic form) was homozygous for the F383Y mutation. Interestingly, case 3, who was a compound heterozygote for E174K and F383Y, exhibited hepatic symptoms. We also identified a polymorphism in the CPTII gene, a phenylalanine (352)-to-cysteine substitution (F352C). According to an expression analysis, this mutation did not alter CPTII activity. It was present in 21 out of 100 normal alleles in the Japanese population, but was not observed among Caucasians. Genotyping with this polymorphism and the previously reported polymorphisms V368I and M647V allowed normal alleles to be classified into five haplotypes. In all three families, the E174K mutation resided only on F1V1M1 allele, while the F383Y mutation was observed on F2V2M1 allele, suggesting a single origin of each mutation.

B-57

MOLECULAR ANALYSIS OF LESCH-NYHAN SYNDROME: A JAPANESE FAMILY WITH TWO POINT MUTATIONS AT INTRON 1 OF HPRT GENE.

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A virtually complete deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT) causes Lesch-Nyhan syndrome. A novel mutation of HPRT gene in a Japanese family has been identified by analysis of the HPRT gene of the patient. Two nucleotide substitutions of G to T at the first nucleotide (M1) and G to T at the eighth nucleotide (M8) from the 5'-end of intron 1 were detected by direct sequencing of PCR products from the patient's genomic DNA. In the patient, an altered mRNA including 49-bp of the 5'-end of intron 1 was expressed. The altered mRNA resulted in small polypeptide consisting of 8 amino acids encoded in HPRT exon 1 and 17 different amino acids. By the expression studies in human HPRT⁻ cells utilizing a mammalian expression vector, respective substitutions (M1, M8 and M1+M8) introduced pCI, it was clarified that the nucleotide substitution at the first nucleotide of 5'-end of intron 1 (M1) caused the splicing error changing the splice donor consensus sequence GT to TT.

B-58

MOLECULAR ANALYSIS OF DAX-1 GENE IN X-LINKED ADRENAL HYPOPLASIA CONGENITA. Kenji FUJIEDA, Jun NAKAE, Toshihiro TAJIMA, Nozomi SHINOHARA, Mari MURASHITA, and Shuji ABE (Dept. Pediatrics, Hokkaido University School of Medicine, Sapporo)

The DAX-1 [DSS (dosage-sensitive sex)-AHC critical region in the X, gene 1] gene has been reported to be responsible for X-linked adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism. However, the function and structure of the DAX-1 protein have not been characterized. In this study, molecular analysis of the DAX-1 gene from 6 patients with AHC, including 2 siblings, identified 5 novel mutations with 3 nonsense mutations and 2 frameshift mutations. Case 1 had a nonsense mutation at position 395 (Q395X). Cases 2 and 3, who were siblings, had a nonsense mutation at position 91 (Y91X). Case 4 had a 2-base deletion (AT) at nucleotide 1610 and 1611 and a 1-base insertion (G) resulting in a premature stop codon at position 462 (1610-1611 del AT ins G). Case 5 had a nonsense mutation at position 271 (Y271X). Case 6 had a 1-base deletion (C) at nucleotide 1169, which induced a frame shift and a premature stop codon at position 371 (1169 del C). All mutated DAX-1 proteins had truncated C-terminal domains. These results suggest that these 5 novel mutations are responsible for X-linked AHC and that the C-terminus of the DAX-1 protein, especially 11 amino acids, is necessary for normal adrenal cortical embryogenesis.

B-59

ANALYSIS OF DAX-1 GENE IN A PATIENT WITH X-LINKED CONGENITAL ADRENAL HYPOPLASIA AND HYPOGONADOTROPIC HYPOGONADISM. Kazuyuki HAMAGUCHI, Masaya ARIKAWA, Seigo YASUNAGA, Tetsuya KAKUMA, Koji FUKAGAWA, Toshije SAKATA (Dept. Int. Med. I, Oita Med. Univ., Oita), Toshihiko YANASE, Hajime NAWATA (Third Dept. Med., Kyushu Univ., Fukuoka).

X-linked congenital adrenal hypoplasia (AHC) is a rare disorder manifesting as primary adrenal insufficiency and hypogonadotropic hypogonadism (HHG). DAX-1 gene has been reported to be a responsible gene. The present case (MK), 20 yr old, is a Japanese male patient. Within a week after birth, MK frequently developed nausea and vomiting. He was diagnosed as adrenal insufficiency, and replacement of hydrocortisone was started. He had no secondary sex characteristics even at age of 18 yr. On admission, he showed generalized hyperpigmentation of the skin. He had no axillary hair and scanty pubic hair. His bilateral testes were atrophic. Bone age was 13 yr 6 mo. Neither adrenal gland was detected by abdominal CT. Endocrinological data were compatible with AHC and HHG. Sequence analysis of MK's DAX-1 gene revealed a 1-bp (G) deletion at amino acid position 49 in exon 1. This mutation yields frame-shift, such that the mutant protein has a completely different amino acid sequence from codon 49 to the premature stop codon at codon 84. His mother was a heterozygote of this mutation. His father and the two younger brothers had normal allele. Pulsatile administration of synthetic LHRH resulted in insufficient secretion of FSH and LH. Therefore, HCG/FSH injection has been started, and the patient is under careful follow up in our hospital.

B-60

GENE ANALYSIS OF 7 JAPANESE FAMILIES WITH HYPOPHOSPHATASIA. Hideo ORIMO¹, Masae GOSEKI-SONE², Tadahiro IIMURA², Yuzo TAKAGI³, Hisashi WATANABE⁴, Kazuhisa TAKETA⁵, Seiji SATO⁶, Hideaki MAYANAGI⁷, Akira OHTAKE⁸, Shinichiro OIDA² and Takashi SHIMADA¹ (¹Dept. Biochem. Molec. Biol., Nippon Med. Sch., Tokyo, ²Dept. Biochem., ³Dept. Pedodontics, ⁴Dept. Periodontology, Faculty of Dentistry, Tokyo Med. Dent. Univ., Tokyo, ⁵Dept. Public Health, Okayama Univ., Okayama, ⁶Dept. Pediatrics, Keio Univ., Tokyo, ⁷Dept. Pedodontics, Tohoku Univ., Sendai, ⁸Dept. Pediatrics, Saitama Med. Sch., Moro)

Hypophosphatasia (HOPS) is an autosomal recessive disease with skeletal hypomineralization due to deficiency of tissue-nonspecific alkaline phosphatase (TNSALP). We screened the TNSALP gene in 7 Japanese families with HOPS using PCR-SSCP-direct sequencing method and found 9 novel mutations. E281K, ΔF310, and frameshifts downstream from S328 and from L503 were found in 3 cases of perinatal/infantile type. F310L and V365I, A160T and F310L were found in a childhood type and an adult type, respectively. A94T, E174G and frameshift from L503 were found in 2 cases of odontohypophosphatasia. Partial intronic sequences of the TNSALP gene were determined to design the PCR primers that amplify whole coding exons. The mutations found in Japanese HOPS were completely different from those in North America and there were no clear relations between the mutation sites and phenotypes.

B-61

PRENATAL DIAGNOSIS OF HYPOPHOSPHATASIA BY PCR-RFLP. Kenji IDA¹, Emiko ABE¹, Shigeo YURA¹, Takafumi NONOGAKI¹, Hiroshi OZASA¹, Norio MIYAMOTO¹, Kenji NAKAMURA², Tsuyoshi TANIMURA², Kenya NISHIOKA², Tomomi NISHIMURA³, Hisako ONO³, Okuyoshi SAIGA³. (Dept. OBGYN¹, Dept. Pediatrics², Dept. Pathology³, OTSU RED CROSS HOSPITAL) Nobuyuki YAMAGISHI, Hiraku TAKEBE. (Dept. Radiation Genetics, KYOTO UNIVERSITY SCHOOL OF MEDICINE, KYOTO)

Prenatal Genetic Diagnosis was attempted to a pregnant woman whose previous infant was diagnosed as perinatal hypophosphatasia. DNA was purified from amniotic cell collected by amniocentesis at 17th week of gestation and from lymphocyte of the parents and the affected baby. Restriction fragment length polymorphism (RFLP) using PCR product of intron 9 of tissue nonspecific alkaline phosphatase gene (TNSALP) digested with Bcl-1 revealed the fetus to be the carrier or affected. The intact development of the bones shown by the fetal sonogram and X-ray study implied the fetus to be the carrier. The pregnancy was continued and the healthy male infant of birthweight 3950g was born with the normal serum-ALP level.

B-62**USE OF BUCCAL SMEARS FOR RAPID DETECTION OF PEROXISOMES.**

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Diagnosis of peroxisome biogenesis disorders such as Zellweger syndrome and neonatal adrenoleukodystrophy has been based on demonstrating the defect of peroxisomes in hepatocytes or fibroblasts. These methods are invasive or time-consuming. We investigated on the possible use of buccal smears for the screening of the Zellweger syndrome. Buccal smears were washed once with saline and spread on the fluorescence-free cover slips. The cells were stained by indirect immunofluorescence staining using antibodies against catalase or acyl-CoA oxidase, and FITC-conjugated goat F(ab')₂ anti-rabbit IgG. Peroxisomes in the control buccal smear cells were visualized with anti-catalase and acyl-CoA oxidase. Peroxisomes were detectable in more than 70% of the smear cells from the control subjects. In two patients with Zellweger syndrome, peroxisomes were not detected using either anti-catalase or anti-acyl-CoA oxidase. Buccal smears are potential materials for rapid and non-invasive diagnosis of peroxisome biogenesis disorders. This method will also be applicable to the diagnosis of other inborn errors of metabolism.

B-63

STRUCTURAL ORGANIZATION AND EXPRESSION OF MANB GENE ENCODING THE HUMAN α -D-MANNOSIDASE. Nobuaki WAKAMATSU, Yasuo GOTODA, Hisaomi KAWAI (First Dept. of Internal Medicine, School of Medicine, The Univ. of Tokushima, Tokushima), Vicki L. Nebes (Allegheny-singer Research Institute, Pittsburgh, PA)

α -D-mannosidase (E.C.3.2.1.24) is a lysosomal hydrolase that catalyzes the hydrolysis of terminal α -D-mannoside residues from N-linked carbohydrates of glycoproteins. To analyze the gene function of this enzyme, we screened human super COS cosmid libraries and isolated four overlapping clones containing α -D-mannosidase (MANB) gene. MANB gene spans approximately 23 kb and consists of 24 exons. The first 160 bp of 5' flanking sequence shows a high G+C content(76%) with two candidate GC boxes and three Ap-2 binding sites. Promoter analysis using CAT reporter system demonstrated that 150 bp of 5' sequence had activity to promote this gene. Thus, like other lysosomal hydrolases, MANB gene is also controlled by sequences located within 150bp of ATG initiation site.

B-64

IDENTIFICATION OF MUTATIONS IN THE *MANB* GENE IN PATIENTS WITH α -MANNOSIDOSIS. Yasuo GOTODA, Nobuaki WAKAMATSU, Hisaomi KAWAI, Yoshihiko NISHIDA, and Shiro SAITO (First Dept. of Internal Med., School of Med., Univ. of Tokushima, Tokushima)

α -Mannosidosis which is characterized clinically by hearing loss, coarse face and mental retardation, is an autosomal recessive lysosomal storage disease with deficiency of α -D-mannosidase activity. We have analyzed the α -D-mannosidase (*MANB*) gene in 6 unrelated type II patients using SSCP to screen all 24 exons and intron/exon boundaries of the gene, followed by direct sequencing and restriction-enzyme analysis of abnormal DNA fragments obtained by the screening. Three of these patients had a homozygous mutation of A146T (His49Leu), C995G (Pro332Arg) and C2176T (Arg726Trp), respectively, while one patient was a compound heterozygote with C1843T (Gln615Stop) and C2176T(Arg726Trp) mutations. No mutations were detected in other 2 patients by the present method. These results indicate that type II α -mannosidosis is mostly due to point mutations of the *MANB* gene.

B-65

A CASE OF MILD TYPE WOLMAN DISEASE

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Wolman disease (WD), characterized by hepatosplenomegaly, steatorrhea, abdominal distention and calcification of adrenal glands, is a rare autosomal recessive disorder and fatal in infancy. WD has been reported to be due to the defect of human lysosomal acid lipase (HLAL). Recently, it is reported that some patients with WD have some mutations of *LIPA* gene, the responsible gene for HLAL. We report here a 3-years-old girl with mild type WD together with the results of *LIPA* gene analysis.

Anemia and liver dysfunction were noticed at the age of 1 month. A marked abdominal distention and hepatosplenomegaly appeared at the age of 3 month. Laboratory data showed hyperlipidemia (TG:448mg/dl, TC:158mg/dl). Abdominal CT scan revealed calcifications of the adrenal glands. Histological examination of the liver showed enlarged and vacuolated hepatic cells. HLAL activity measured from peripheral lymphocytes was extremely reduced. She was diagnosed as having WD and has been treated with low fat diet and the HMG-CoA reductase inhibitor.

LIPA gene of the patient was analyzed by using exon specific PCR-SSCP. PCR product amplified with exon 1 specific primers indicated divergent SSCP band. Further analysis with sequencing would elucidate the phenotype-genotype correlation of mild type WD.

B-66

RELATIVE FREQUENCY OF ANGELMAN SYNDROME IMPRINTING MUTATION PATIENTS. Takahito WADA¹, Shinji SAITOH¹, Naomichi MATSUMOTO², Norio NIIKAWA² (1Dept. Pediatr. Hokkaido Univ. Sch. Med., Sapporo; 2Dept. Hum. Genet. Nagasaki Univ. Sch. Med., Nagasaki)

Angelman syndrome is a neurobehavioral syndrome characterized by severe mental retardation, epilepsy, characteristic facies, and happy disposition. Incidence is estimated as 1/10,000 to 1/20,000, and it is one of common genetic disorders in severely retarded patients.

Approximately 70 % of AS patients have a large deletion of maternally inherited 15q11-q13, which is easily detected by FISH. Less than 2 % of AS patients have paternal uniparental disomy (UPD) of chromosome 15. Rest of patients are designated as non-deletion, non-UPD patients. Recently, a new subset of AS patients are identified, who have DNA methylation abnormality throughout 15q11-q13, and named imprinting mutation (IM) patients.

We previously examined 61 AS patients, and 66 % showed a large deletion, none had UPD, and 34 % were non-deletion, non-UPD patients. To know the frequency of IM patients, we performed DNA methylation tests using SNRPN and PW71 as probes in 17 non-deletion, non-UPD patients (4 familial and 13 sporadic), whose DNA were available from the previous study. Paternal only DNA methylation pattern (AS pattern) was identified in 2 familial (2/4) and 2 sporadic (2/13) patients. These findings clearly indicate that IM is not rare even in sporadic patients. Since most of IM patients are known to have inherited small deletions at Imprinting center (IC) region, it is important to identify IM patients for genetic counseling purpose.

B-67

A MOLECULAR STUDY OF ANGELMAN AND PRADER-WILLI IMPRINTING MUTATION PATIENTS. Shinji SAITOH¹, Robert D. NICHOLLS² (1Dept. Pediatr. Hokkaido Univ. Sch. Med., Sapporo; 2Dept. Genet. Case Western Reserve Univ., Cleveland, USA)

Imprinting mutation is a newly identified subset of patients of Angelman (AS) and Prader-Willi (PWS) syndromes. It is characterized by abnormal DNA methylation throughout 15q11-q13 despite no evidence of a typical deletion or uniparental disomy (UPD). It is believed to be associated with a mutation of imprinting process.

We examined six AS and three PWS imprinting mutation families. A typical deletion and UPD were ruled out by FISH and PCR microsatellite analyses. DNA methylation tests at SNRPN, D15S9, and D15S63 loci revealed paternal only methylation pattern in all of AS patients, and maternal only pattern in all of PWS patients examined. Expression study of SNRPN, IPW, and ZNF127 genes showed no expression of any genes in PWS imprinting mutation patients, whereas it showed biallelic expression in AS imprinting mutation patients.

We used a phage contig covering D15S63 through SNRPN and identified small deletions (6 to 150 kb) located upstream of the SNRPN gene in these patients. The same deletions were found in their mothers and maternal grand fathers (AS); and in their fathers and paternal grand mothers (PWS). Therefore, this region, we designated imprinting center (IC), should contain a novel *cis* acting genetic element which plays a role in erasing or resetting of genomic imprinting of 15q11-q13 during gametogenesis.

B-68

Molecular mechanism of genomic imprinting in the region of human SNRPN gene. Mitsuyoshi NAKAO(Tumor Genet. and Biol., Kumamoto University, Kumamoto), Ikuko KONDO(Hygiene, Ehime University, Matsuyama), Hug. A.H.M.M. and Arthur L. BEAUDET(Molec. and Hum. Genet., Baylor College of Med., Houston)

The gene encoding for the small nuclear ribonucleoprotein-associated polypeptide N(SNRPN) localizes in the Prader-Willi syndrome(PWS) critical region on chromosome 15, and is expressed only from the paternal allele. A CpG island containing the exon α of SNRPN gene is differentially methylated on the maternal chromosome. Sequence analysis of cosmid 102 containing of exon α , β and 1 as well as 20 kb-upstream from exon α revealed there are multiple direct repeat sequences ~0.8 kb-downstream exon α which are thought as character of the imprinted genes. A DNaseI-hypersensitive site was also found ~0.7 kb-upstream exon α . The promoter of exon α showed a much stronger activity than that of exon 1, and was completely abolished by in vitro methylation. These findings suggest that the imprinted regulation of SNRPN gene is correlated with short direct repeats, open chromatin structure and DNA methylation. Since recent reports of several imprinting mutations in the PWS patients with smaller deletions have suggest an imprinting center in male germline is close to exon α , our data will be very helpful for the identification of such regulatory elements in this region.

B-69

THE MOUSE *L23mrp* GENE LOCATED DOWNSTREAM OF THE IMPRINTED *Ins2/Igf2/H19* REGION IS BIALLELICALLY EXPRESSED AND NOT INFLUENCED BY THE *H19* ENHANCER. Hiroyuki SASAKI and Mohamad ZUBAIR (Institute of Genetic Information, Kyushu University, Fukuoka)

The *Ins2* (insulin II), *Igf2* (insulin-like growth factor II) and *H19* genes are closely linked on mouse chromosome 7 and all are subject to tissue-specific parental imprinting. It was previously shown that the imprinting of these genes involves their parental-origin-specific interactions with the endoderm-specific *H19* enhancer. To know the extent and boundaries of this imprinted domain, we have cloned and characterized the mouse homolog of the human *L23MRP* gene, which had been mapped approximately 40 kb downstream of the human *H19* gene. Our findings show that the mouse *L23mrp* gene is expressed biallelically in various developmental stages and tissues. On the basis of the analysis of *H19* enhancer deletion mice, we also conclude that the enhancer does not influence *L23mrp* expression. Thus it is likely that this gene demarcates the 3' end of the imprinted domain. The lack of interactions between the enhancer and the gene may be due to either the promoter specificity of the enhancer or to the presence of a putative chromatin insulator.

B-70

**GENOMIC CLONING AND LOCALIZATION OF THE HUMAN
ACHAETE-SCUTE HOMOLOG 2 (*ASCL2*) TO CHROMOSOME 11p15.5**

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The mouse achaete-scute homolog-2 gene (*Mash2* or *Ascl2*), a member of the achaete-scute gene family, encodes a transcription factor that plays an important role in the development of the trophoblast. It has recently been reported that mouse *Ascl2* is genomically imprinted and mapped to the distal region of mouse chromosome 7, where other imprinted genes are located. We isolated a clone carrying the partial human homolog, *ASCL2*, from a human genomic phage library using a PCR product for the rat *Ascl2*-cDNA as a probe. Sequence comparisons of the human *ASCL2* clone and the rat *Ascl2*-cDNA showed 86.9% identity spanning 206 nucleotides. By FISH using a phage clone as a probe, we assigned *ASCL2* to human chromosome 11p15.5, where the human imprinted gene, *p57^{KIP2}*, *IGF2* and *H19* are located. Thus, it is of great interest to know whether *ASCL2* is also imprinted.

B-71

**IDENTIFICATION AND STRUCTURAL ANALYSIS OF A REGION WITH A
PATERNAL-SPECIFIC METHYLATION IMPRINT AT THE HUMAN H19 LOCUS.**

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We have identified a region with characteristics of a paternal-specific methylation imprint at the human H19 locus. This region, extending from -2.0 kb upstream to the start of transcription, is heavily methylated in sperm and on the paternal allele in somatic cells. This methylation was preserved during pre-implantation. A structural analysis revealed a feature of CpG islands and a striking organization of a large direct repeat with a 400 bp sequence reiterated several times, but no significant sequence homology to the corresponding region of the mouse H19 gene. While these findings present somewhat puzzling aspect as to evolution of the H19 regulatory region in human and mouse, they may explain the apparent difference in H19 expression in human placenta compared to mouse.

B-72

ISSUES SURROUNDING GENETIC DISEASE DISCRIMINATION. Mitsushiro KIDA (Teikyo Women's Junior College)

One university professor has said, "In today's society, smaller numbers of babies are being born and smaller numbers of people are dying. Genetic mutation is thus something that cannot be avoided. If selection is at work, as it was in Japan in the past, genetic abnormalities will not accumulate in society, but they will naturally accumulate in today's society.

As a result, by conducting genetic tests, abortions should be performed, otherwise society as we know it will cease to function. "He advocates the spread of genetic tests before birth and unconditional artificial abortion when a genetic abnormality is detected. After lecturing for a while at a women's college, I asked the students two true/false questions: (1) Both of my parents are healthy and all of my blood relatives are healthy. Thus, I have no bad genes. (2) If all people with a genetic disease decided not to have children, then there would be no-one on earth with a genetic disease. They are both "false." The ratio of correct answers for question 1 was 254/377 (67.3%); for question 2, 314/377 (83.8%).

These two examples shine light on the ethical problems that human geneticists of today avoid and will not discuss, such as, who decides the right and wrongs of a particular disease? when, and with what authority?; Are all genetic diseases bad and must be eradicated? Will it be possible to eradicate all genetic diseases by artificial selection? Should a society force artificial abortion on people?; Should such question be decided only by specialists and public officials without counseling the general public, including the patients themselves? The idea of eugenics is a concept put by Galton in England in 1885, and was so abused by Hitler. Human genome researchers must not travel the same path.

B-73

CONSENSUS SURVEY ON HEREDITY IN FUKUI, INDIA, BEIJING. Mikio HIRAYAMA, Masaru KURIYAMA (Second Dept. Int. Med., Fukui Medical School, Fukui), Norio FUJIKI (Toyobo Gene Lab. Tsuruga), Shigeaki NAKAZAKI (Kunitaka Hospital, Takefu), L.C. VERMA (Dept. Pediat. All India Inst. Med. Sci., New Delhi) and Wilson H. Y. LO (Inst. Basic Med. Sci. Peking Union Med. Coll, Beijing)

Consensus survey was done in Fukui, India, Beijing. Those with an image of heredity as scientific were 26% in Fukui, 63% in India, 80% in Beijing. 3% answered correctly the frequency of congenital anomaly in Fukui, 24% in India, 37% in Beijing. Knowledge of prenatal diagnosis was 40% in Fukui, 63% in India, 75% in Beijing. 74% would take the amniocentesis test in case of Down's syndrome in Fukui, 43% in India, 80% in Beijing. Those who would abort the child after the result was positive, were 40% in Fukui, 70% in India, 87% in Beijing. In Beijing, most of the respondents were with higher education and one child policy increased the knowledge of genetic diseases. In India, most of the respondents had handicapped children, and this influenced the result. The necessity of education of medical genetics was emphasized.

B-74

SHINSHU UNIVERSITY HOSPITAL ESTABLISHED THE DIVISION OF CLINICAL GENETICS AS ONE OF ITS CENTRAL SERVICE DEPARTMENTS. Yoshimitsu FUKUSHIMA, Shuichi IKEDA, Koyo SAKURAI, Kenichi KOIKE, Kazuhiko MATSUMOTO, Minoru FUJIMORI, Toshio NIKAIDO, Kenichi FURIHATA, Kiyoshi MATUO and Jun KOMIYAMA (Div. Clin. Genet., Shinshu University Hospital)

Recent advances in medical genetics enable us to do carrier detection, presymptomatic diagnosis or prenatal diagnosis of many genetic disorders. These techniques are useful for reduction of anxiety of patients or families, and early diagnosis and treatment. To effectively use these techniques, a division of clinical genetics, at which genetic diagnosis and counseling are provided, is necessary. However, the field of clinical genetics is lagging in Japan. Shinshu University Hospital established the division of clinical genetics as one of its central service departments. Several MDs from the departments of internal medicine, geriatrics, pediatrics, surgery, laboratory medicine and hygiene staff this project. All of the staff are certified as members of the Japanese Board of Medical Genetics, Clinical Geneticist or as its trainees, and serve concurrently. This is the first clinical genetics department in the National University Hospitals in Japan. This system of having a clinical genetics division is likely to be available in other university hospitals, while some problems lie in the referring system, confidentiality and financial support.

B-75

ATTITUDE OF YOUNG WOMEN STUDYING HEALTH CARE TO PRENATAL MATERNAL SERUM SCREENING. Tomoko HASEGAWA, Chigusa MASUMURA, Kimiko MIYAKAWA, Tomoyuki KATAHIRA (Div. Clin. Genet., Shizuoka Children's Hospital, Shizuoka, Dept. Human Life & Environmental Health, Niigata Women's College, Niigata, Dept. Gynecol. & Obstet. National Nagoya Hospital, Nagoya)

Prenatal maternal serum (MS) screening for Down syndrome (DS) has recently started in Japan. However many medical scientists do not agree with the spread of this screening, since it is easy to perform in any obstetric clinic, but genetic counselling staff are not available at most of these clinics in Japan. In April 1996 a documentary about MS screening was televised. We asked female students taking a midwifery training course at two nursing schools, and a social welfare course at a college for their opinion about this screening after watching the TV programme. Most of the students were unable to comprehend exactly the concept of MS screening. However, they were concerned that as the results of screening is not certain, it is difficult for the public to understand, therefore women who obtain a positive or false negative result may be psychologically injured. They were also concerned that pregnant women might select easy termination of pregnancy without considering the life and rights of the people with DS. During lectures on congenital disorders, students should be taught that most parents have a great love for their children with DS and feel that the life including people with DS in the family and the community is not only suffering but also pleasing. However, only a few people get this information. We asked 20 students at the nursing school in Shizuoka whether they would conduct screening or not, and 15 stated that they would not. Four stated that they would conduct it. Of these, three stated that they would wish to know and think of the life of their future children and themselves, and one stated that she would terminate for fear of child with any anomaly if the result of the screening was positive.

B-76

Mutation View : A NETWORK DATABASE SYSTEM FOR HUMAN DISEASE-ASSOCIATED MUTATIONS. Shinsei MINOSHIMA, Susumu MITSUYAMA and Nobuyoshi SHIMIZU (Department of Molecular Biology, Keio University School of Medicine, Tokyo)

The genes responsible for hereditary disorders and cancers are being investigated as one of the most important aspects of the human genome project. To date, approximately 1,100 diseases have been mapped to particular chromosomal regions and over 500 disease-causing genes have been identified. We have developed a computer software *Mutation View* to systematically integrate genetic, molecular biological and clinical findings of each disease and to assist DNA diagnosis and gene therapy. The features of *Mutation View* are as follows.

(1) Chromosome ideograms are drawn to list diseases in the mapped regions. OMIM documents can be cited for each disease. (2) Primary structure of the gene or its cDNA is shown schematically and various mutations are located on appropriate positions with symbols. The detailed information of each mutation is displayed by clicking of the mutation symbol. Zoom-in is possible in wide ranges until nucleotide sequence can be displayed. Relative frequency or number of cases for each mutation can be shown as a histogram and classification of mutation types or symptoms is available. (3) Input and editing of new mutations are easily manipulated and new data can be shown together with existing data. (4) The exact mutation is shown with reference of normal nucleotide sequence. Change of restriction sites on the mutation position can be automatically analyzed even for newly added data. (5) Since this *Mutation View* has been designed as a distributed database, any laboratory can take a role as a server site for a particular disease and the users can access to any disease information *via* internet.

A prototype of *Mutation View* using a cystic fibrosis database compiled by Dr. Lap-Chee Tsui has been demonstrated using a workstation.
(This work was done in collaboration with Chi Co., Ltd. and NEC Corporation.)

C-1

PRESSURE-INDUCED CELL DEATH AND APOPTOSIS IN HUMAN LYMPHOBLASTS. Takako TAKANO, Yasuko YAMANOUCI (Dept. of Hygiene & Pub. Health, Teikyo Univ. School of Med., Tokyo), Takaaki SATOU and Kaoru J. TAKANO (Institute of Materials Science, Univ. of Tsukuba, Tsukuba)

The observation of human cells at high pressure is useful to research the cell biology and medical application. We investigated the cell death of EB virus transformed human lymphoblast pressureized for 30 minutes at 37°C using a high pressure apparatus. The viability just after releasing high pressure decreased from 100% to 0% with increasing pressure from 85 MPa to 200 MPa. In the case of 50 MPa, the viability was not damaged during monitored 48 hours after decompression and cell counts increased gradually. In the case of 100 MPa, necrosis progressed after decompression for about 12 hours. The apoptotic cells, which were detected by TUNEL method and Hoechst 33258 nuclear stain, began to be observed from 8 hours after decompression. By 24 hours, there was an increase in the percentage of apoptosis. Induction of apoptosis by compression and acceleration of both apoptosis and necrosis in S phase, were confirmed.

C-2

EVOLUTIONARY CONSIDERATION FOR THE GENEALOGICAL ORIGIN OF HUMAN CHROMOSOME 9 BY FISH ANALYSES IN HIGHER PRIMATES. Hideyuki TANABE¹, Takafumi ISHIDA², Shintaroh UEDA², Toshio SOFUNI¹ and Hiroshi MIZUSAWA¹ (¹Cell Bank Labo., Div. Genet. and Mutagenesis, Natl. Inst. Health Sci., Tokyo, ²Graduate School of Sci., The Univ. of Tokyo, Tokyo)

Evolutionary origin of human chromosome 9 (HSA9) and its homologues of higher primates were genealogically considered through comparative mapping of the immunoglobulin processed pseudogene Cε3 (IGHEP2) and three DNA markers on HSA9. IGHEP2 (HSA9p24.2→p24.1) was assigned to PTR11q34 (common chimpanzee), PPA11q34 (pygmy chimpanzee), GGO13q22 (gorilla), PPY13q16 (orangutan), HLA8qter (white-handed gibbon), HAG8qter (agile gibbon), and MFU14q22 (Japanese macaque) and other DNA markers were assigned to PTR/PPA11p11 (cCI9-37 and 135), PTR/PPA11q22 (cCI9-208), PPY13q22 (cCI9-37 and 135), and PPY13q12 (cCI9-208) by FISH. These results indicate that the pericentric inversions occurred twice during the hominoid evolution generating human and the great ape species. The first pericentric inversion occurred at PPY13p13/13q16 in a human-chimp ancestor after the branching off of the gorilla and the second pericentric inversion took place at PTR11p11/11q22 in the chimp branch. These data provide a hypothesis that supports the results from banding analyses of HSA9 and its homologues.

C-3

AN IMPROVED SILVER-STAINING METHOD FOR "ACTIVE" CENTROMERES: ANALYSIS USING CONSTITUTIONAL DICENTRIC CHROMOSOMES. Jia ZHAO, Tomohiro HAYAKAWA, and Tatsuro IKEUCHI (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The centromere dot (Cd)-staining method by Eiberg (1974) has been well-known to selectively stain the "active" centromere, as revealed with pseudodicentric chromosomes. However, there are some problems in the Cd-staining method for its practical use, including stainability and reproducibility. We have devised a more simplified and reliable method for Cd-staining by modifying the silver-staining technique that is known to detect the nucleolar organizing regions: The method consists of incubation of chromosome slides in 2×SSC at 60°C for 15 min, and staining with an AgNO₃ solution (0.5g of AgNO₃ in 1 ml of DW) covered with a nylon cloth at 80°C for 40-60 min. This Ag/Cd-staining method was applied to two different constitutional dicentric chromosomes seen in phenotypically abnormal patients. In psu dic(18) (p11.32) chromosomes in one case, the Ag-stained Cd was positive only at the primary constriction, and no stain was seen at the second centromeric region, where both the C-band and FISH signals by using an alpha satellite DNA probe were positive with two separate dots on each chromatid. Thus, the present method was confirmed to discriminate between the "active" and "suppressed" centromeres in psu dic chromosomes. The other case had a rob(21;22) (p11;p11) chromosome with two C-bands, in which the two pairs of silver-stained Cd were detected, indicating that the two centromeres in this case were both "active".

C-4

MEIOTIC CROSSING-OVER AND SEGREGATION OF INVERSION CHROMOSOMES. Shin-ichi SONTA, Noriko NOMURA (Department of Genetics, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi)

In the human population, there are a number of inversion carriers. However, the data on the crossing-over and segregation of inversion chromosomes are insufficient. By chromosome analysis of meiotic cells in male and female Chinese hamsters heterozygous for inversion 1, 4 and X, we examined the segregation, meiotic crossing-over on the interstitial segment and interchromosomal effect of inversions. The chromosome analysis of MII cells revealed that the number of cells with an inversion and ones with a normal homologues was almost equal. The crossing-over on the interstitial segments of inversion chromosomes caused the appearance of a marker chromosome with unequal-length chromatids at MII. The analysis using such marker chromosomes revealed that the frequency of cells with crossing-over on the interstitial segments of inv(1), inv(4) and inv(X) chromosomes in female heterozygotes was 18.2%, 5.5% and 1.2% which was significantly lower than that on the same segments of normal chromosomes 1, 4 and X. Further analysis of MII chromosomes from single and double heterozygotes for both inversion and reciprocal translocations indicated that the existence of inversion induced no abnormal disjunction of normal chromosomes and also had no influence on the segregation of the quadrivalents. These findings suggest that inversions have no interchromosomal effect.

C-5

STUDY OF ANEUPLOIDY RATE OF SPERM IN A MAN HETEROZYGOUS FOR A t(3;9)(p25;q32) RECIPROCAL TRANSLOCATION BY FISH. Hiroshi HONDA, Norio MIHARU, Nao NAKATA, and Koso OHAMA. (Dept. of Obstet.and Gynecol., Hiroshima Univ. School of Medicine, Hiroshima)

[Objectives] In order to quantify the relative proportion of sperm with unbalanced chromosomes resulting from the meiotic segregation, molecular cytogenetic analysis was done with sperm from a man heterozygous for a t(3;9)(p25;q32) reciprocal translocation carrier using fluorescence in situ hybridization (FISH). [Methods] Semen samples were obtained from the carrier and a normal man. Two color FISH was performed with DNA probes (biotin labeled alpha-satellite D3Z1 probe and digoxigenin labeled beta-satellite D9Z5 probe). A total of 3319 sperm from the carrier and 3050 sperm from normal man were scored. [Results] The frequency of sperm with (3/-), (3/9), (3/3/-), (3/3/9), (3/3/9/9), (3/9/9), (-/9/9), (-/9) signal was 2.95%, 90.3%, 0.271%, 1.48%, 0.181%, 1.17%, 0.421%, 3.10%, respectively in a carrier, and 0.280%, 99.0%, 0%, 0.280%, 0.0628%, 0.249%, 0%, 0.0621% in a control man. [Conclusion] These data indicate that at least 10% sperm are produced by unbalanced segregation.

C-6

OBSERVATIONS OF DERIVATIVE CHROMOSOMES FROM RECIPROCAL TRANSLOCATIONS BY SCANNING ELECTRON MICROSCOPE.

Takenari NIINUMA, Shigeki UEHARA, Toshifumi TAKABAYASHI, Kunihiro OKAMURA, Akira YAJIMA. (Dept. Obstet. Gyne. Tohoku Univ. Sendai.)
Hiroyuki SASAKI (SASAKI Gyne. Sendai)

we observed the fine structure of derivative chromosomes from reciprocal translocations by scanning electron microscope (SEM) and recognized irregularity of the chromosomal structure on the connective points of reciprocal translocations. we studied three cases: 46,XX,t(6;7)(q25;p15), 46,XX,t(X;19)(q22;q13), 46,XX,t(3;14)(p21;q32) and had white blood cells from them. we cultured them on RPMI 1640 (20% FCS, 3% PHA added) for 72 hours and added Colcemid and harvested and made them low tensile and fixed dry on slideglasses. we dyed them by Gyemsa method for 3 hours and took photographs by light microscope and choose adequate chromosomes on metaphase and cut and put them steamed by platinum and observed by SEM. we recognized gap which width is about 0.5 μ m in correspondence to the points connected by large fragments of reciprocal translocations (der(6), der(19), der(14)) in all three cases and we did not find out the same changes of structure in derivative chromosomes connected by small fragments of reciprocal translocations. this data suggest that there is the irregularity of chromatin fibers in connective points of derivative chromosomes originated from reciprocal translocations and the width is in accordance with middle sized G-band.

C-7

PCR-BASED DETECTION OF MOSAICISM IN CYTOGENITICALLY 45,X TURNER SYNDROME PATIENTS. Tohru YORIFUJI, Junko MUROI, Masahiko KAWAI (Dept. Pediat., Kyoto University Hospital) Hiroshi SASAKI (Dept. Pediat., Kurashiki Central Hospital), Toru MOMOI (Dept. Pediat., Japanese Red Cross Society Wakayama Medical Center), Masayuki KAJI (Dept. Pediat., Shizuoka Children's Hospital),

To study the possible role of cryptic mosaicism in phenotypical variations of 45,X Turner syndrome, we analyzed low-level mosaicism by PCR-based methods. For detection of X chromosome mosaicism, we employed a novel modified HUMARA assay, which proved to be a sensitive method with a detection limit as low as 1 in 960 cells. Using this assay, we detected low frequency cryptic X chromosome mosaicism in 2 of 18 cytogenetically 45,X patients.

C-8

SMALL INTERSTITIAL DELETION OF THE LONG ARM OF CHROMOSOME 2 (2q24.3) DETECT BY ASSISTANCE WITH OUR ORIGINAL COMPUTERIZED DATABASE, UR-DBMS. Yasutsugu CHINEN, Takaya TOHMA, Yoshinori IZUMIKAWA, Tetsu IHA, Yoshinobu GOYA, Kenji NARITOMI (Dept. Pediat., Ryukyus Univ.Sch. Med.,Okinawa)

To date, 30 patients have been described with deletions of the long arm of chromosome 2. A distinct phenotype-karyotype correlation has been suggested in patients with del(2)(q31q33)(Ramer et al., 1989). We report on an additional patient with a karyotype, 46,XX,del(2)(q24.2q31). The proposita was born at 38 weeks of gestation and had a birth weight of 1984g. She had a peculiar facies similar to Treacher-Collins syndrome due to downward slanting of the palpebral fissures, low-set and posteriorly rotated ears and microretrognathia. Other findings included an occipital encephalocele, hypertelorism, short palpebral fissures, a beaked nose with its bulbous tip, clenched hands and complete atrio-ventricular septal defect. At two months of age, she died of the progressive heart failure. By the use of our original computerized database, UR-DBMS (Naritomi, 1996), chromosomal aberrations were highly suspected. We detected a small interstitial deletion involving the band 2q24.3 in high-resolution GTG-banded chromosomes. To confirm a phenotype-karyotype correlation among medial 2q monosomies, clinical findings of 10 reported patients with a deletion involving 2q24 were compared with those of 11 reported patients with a del(2)(q31q33). As a result, the cardinal findings and their frequencies are almost similar between the two. Because the smallest region of overlap is located at a band 2q31, we may define a 2q medial monosomy syndrome associated with a deletion of 2q31.

C-9

SEX PHENOTYPE IN 45,X/46,X,dic(Yq) INDIVIDUALS: CYTOGENETIC AND MOLECULAR STUDIES. Michio TERAOKA, Koji NARAHARA, Kiyoko KOYAMA, Yuji YOKOYAMA, Rintaro Mori and Yoshiki SEINO (Dept. Pediatr. Okayama Univ. Med. Sch. Okayama)

The 45,X/46,X,dic(Yq) mosaicism is associated with various sex phenotypes. To clarify whether the sex phenotype is caused by a dilution effect or abnormality of SRY gene, we conducted cytogenetic and molecular studies in 4 patients with this mosaicism, of whom 2 had normal male phenotype and 2 Turner syndrome. Chromosome analysis showed that the ratio of cells with a dic(Yq) varied from an individual to an individual (25-100 % in peripheral blood lymphocytes, and 0-50 % in skin fibroblasts) and was not related to the given sex phenotype. PCR of lymphocyte DNAs demonstrated an intact SRY gene in all patients. FISH studies using a SRY gene probe, pHu14, showed two discrete signals on dic(Yq) chromosomes from both patients with Turner syndrome and male phenotype. We conclude that the distribution of cells with a dic(Yq) in primordial gonads may be a major determinant of sex phenotype in 45,X/46,X,dic(Yq) individuals.

C-10

A CASE OF 45,X/46,X,idic(Y)(q11.2) WITH SHORT STATURE AND NORMAL MALE EXTERNAL GENITALIA.

Eiko HIDAKA¹, Keiko WAKUI², Masayo ISHIKAWA¹, Kyoko ADACHI³, Hideo USHIKU³, Keiko TOMITA⁴, Yutaka NAKAHORI⁴, Tutomu KATSUYAMA⁵, Yoshimitsu FUKUSHIMA² (1Central Clinical Laboratories, 2 Hygiene and Medical Genetics, 3Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, 3Saku General Hospital, 4Tokyo University.)

A 4-month-old male infant was the second child of phenotypically normal parents. He had short stature, normal male external genitalia, and peculiar facies resembling Turner syndrome. Chromosome analyses by G- and Q-banding and FISH using DYZ3 revealed the following karyotype: 45,X[25]/46,X,idic(Y)(q11.2).ish idic(Y)(DYZ3++)[23]/47,X,idic(Y)(q11.2)x2ish idic(Y)(DYZ3++x2)[2]. PCR analysis with Y-specific sequence tagged sites revealed: PABY(+), SRY(+), AMGL(+), DYZ3(+), DYS139(+), DYS132(+), DYS1(-), and DYZ1(-). Thus, the breakpoint was delimited between DYS132 and DYS1.

45,X/46,X,idic(Y)(q11.2) has been reported in more than 70 patients. About a half of the patients were phenotypically female, 25% had ambiguous genitalia and 25% were phenotypically male. The majority of the phenotypically male patients were affected with abnormal genitalia. An estimation of gonadal function, observation of Turner stigmata, DNA analysis of the deleted region of the Y chromosome and detection of mosaic frequency among different tissues are necessary in this patient.

C-11

A FEMALE CASE OF ALPORT SYNDROME COMPLICATED WITH SHORT STATURE AND HYPOGONADISM SHOWING THE KARYOTYPE OF 46X,t(X;1)(q22;p31.1).Makoto INOUE, Satomi MIYAMOTO, Seiji YAMAGUCHI(Dept. Pediatr. Shimane Medical University, Shimane) and Kiyoshi KIKUCHI(Dept. Pediatr. Shimane Prefectural Central Hospital, Shimane)

Alport syndrome is characterized by progressive hereditary nephritis and sensorineural deafness, and is inherited in an X-linked fashion in most patients. It has been attributed to abnormalities in the alpha 5 chain of collagen type IV molecules(COL4A5), whose gene is assigned to chromosome Xq22.

We described a female case with Alport syndrome complicated with short stature(-3SD), obesity(+69%), webbed neck and hypogonadism, showing the karyotype of 46X,t(X;1)(q22;p31.1). It was observed by the R-band staining that normal X-chromosomes were inactivated. Immunohistochemical study of renal tissue revealed undetectable COL4A5. The break point on the X chromosome in this patient was co-incident with the locus of the Alport gene. Moreover, it is located within the gene region of Xq13-27 that participates in the development of gonads and could be causative of Turner syndrome-like symptoms in this patient.

C-12

PALATAL RUGA ANOMALIES IN MUTANT MOUSE AND RAT MODELS FOR HUMAN CONGENITAL ANOMALIES. Mineo YASUDA, Mizuyo TSUGANE, Ichita MIWA (Dept. Anat. Hiroshima Univ. Sch. Med., Hiroshima), Shinichi MIYABARA (Fetal Genetic Pathology Lab., Saga Med. Sch., Saga) and Michio FUJIWARA (Safety Res. Lab., Yamanouchi Pharm. Co. Ltd., Tokyo)

The oral surface of the palate in mice and rats has eight or nine pairs of transverse ridges, or rugae. In the present study, we examined palatal ruga anomalies in trisomy 16 mice (a mouse model for human trisomy 21), trisomy 14 mice (with high incidence of cardiac anomalies), and Uchida rats (a mutant of which heterozygotes have small eyes resulting from a mutation in the Pax-6 gene). All of the trisomy 16 mouse fetuses had severely altered ruga patterns such as deletion, median division, shortness, and maldirection. Trisomy 14 mouse fetuses showed mild anomalies with supernumerary rugae in the posterior part of the palate. Uchida rat young had rugae with division, peakless, discordance, and supernumerary. These findings indicate that the pattern of palatal rugae is a good phenotypic marker for observation of mutant mice and rats.

C-13

DERMATOGLYPHICS ON VOLAR SKIN OF TRISOMY 16 MOUSE FETUSES. Mizuyo H. TSUGANE, Mineo YASUDA (Dept. Anat. Hiroshima Univ. Sch. Med., Hiroshima), Shinichi MIYABARA (Fetal Genetic Pathology Lab., Saga Med. Sch., Saga)

It is widely known that Down's syndrome (human trisomy 21) exhibits characteristic dermatoglyphs. We investigated the dermatoglyphs on volar skin of Trisomy 16 (Ts 16) mouse fetuses, the animal model for human trisomy 21, on gestational day (GD) 18 (plug = GD 0) to clarify the relationship between the trisomy and the abnormal dermatoglyphs. Additionally, we compared the dermatoglyphs of Ts 16 fetuses with those of fetuses maternally treated with all trans-retinoic acid (RA) on GD 12.5.

On the volar skin, normal mice have four kinds of pads; 1) digital, 2) interdigital and carpal (tarsal), 3) caterpillar, and 4) cobblestone pads. The pad pattern forms a characteristic dermatoglyph, and is almost constant in every normal mouse. In Ts 16 fetuses, the pad pattern was affected; the cobblestone pads disappeared on all limbs, extra pads often appeared between interdigital pads, and some hindlimbs lacked the fibular tarsal pad. Similar abnormal pad patterns were observed between Ts 16 and RA-treated fetuses. This is the first time that mouse fetuses with chromosomal aberrations are proved to have abnormal dermatoglyphs as human counterparts. These results suggest the possibility to clarify the mechanisms for abnormal dermatoglyphs due to chromosomal aberrations.

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C-14

THE DIFFERENTIATION OF THE UPPER-LIMB MOVEMENT AND HAND DOMINANCE FOR DOWN SYNDROME. Asagi YAGINUMA, Hiroshi USHIZIMA (Faculty of Medicine, Department of Maternal and Child Health, Tokyo University, Tokyo) and Makoto HIGURASHI (Tokyo Kasei University, Tokyo)

The purpose of this study was to investigate the differentiation of the Upper-Limb movement for children with Down Syndrome. The task of the winding thread around a spool was administered to both Down Syndrome children and comparison groups. Children with Down Syndrome had the retardation of the differentiation of the Upper-Limb movement, and the level of motor development was nearly the same developmental stage as the normal children (4 years to 6 years). Girls with Down Syndrome wended the thread faster than boys with Down Syndrome significantly. Boys wended faster than the right hand than the left hand .

C-15

THYROID FUNCTIONS OF DOWN SYNDROME. Ryuichi TSUKINO (Dept. Pediat. ,Arida City Hospital, Wakayama), Mitsuyo SASAKI (Dept. Pediat. , Wakayama Medical College, Wakayama).

A total of 144 individuals with Down syndrome aged 0 to 29 were observed for medical and social care at genetic department of Wakayama Medical College and Kinan general hospital . As one of these medical cares, TSH, free T3 and free T4 were examined as screening test for thyroid functions. Under one age, about 1/3 showed abnormally high value of TSH ($> 5 \mu\text{U/ml}$). And 3 infants were diagnosed as hypothyroidism and suffered supplement therapy . But the remains were observed without therapy about 6 months for a regular check-up. To evaluate this procedure which proper or not, the height as an index of thyroid functions of two groups (TSH $< 5 \mu\text{U/ml}$ and TSH $\geq 10 \mu\text{U/ml}$ group) were compared. In one group which was observed from under one year , individuals of showing of TSH $< 5 \mu\text{U/ml}$ and TSH $\geq 10 \mu\text{U/ml}$ were 16 respectively. The other group was observed through whole follow -up period and the former was 27 and later 25 respectively. The t test showed no significance difference at height between both groups. We conclude that if abnormal clinical findings and any other abnormal thyroid function tests are not found, this our follow-up studies were proper.

C-16

STUDY ON THE RELATIONSHIP BETWEEN CLINICAL PHENOTYPES AND X CHROMOSOME ABNORMALITIES. Yuko SHINTAKU (Dept. Obstet. Gynecol., Iwate Prefectural Iwai Hospital, Iwate) Shigeki UEHARA, Toshifumi TAKABAYASHI, and Akira YAJIMA (Dept. Obstet. Gynecol., Tohoku University School of Medicine, Sendai)

We have examined X chromosome abnormalities in patients referred to Tohoku University Hospital because of primary or secondary amenorrhea and habitual abortion. 20 of 68 primary amenorrhea (28%), 4 of 16 secondary amenorrhea (25%), and 13 of 266 habitual abortion (5%) showed X chromosome abnormalities. There was 10 of non-mosaics, and 27 of mosaicism. In non-mosaic cases, monosomy X (45,X) was seen in only one case, the other cases had a variety of karyotypes with a structural alteration of the X chromosome: 2 of isochromosome for long arm of X, 3 deletions of part of Xp or Xq, 1 ring X chromosome, 2 balanced translocations and 1 unbalanced translocation. Most of the mosaic cases had 45,X. In habitual abortion 47,XXX, 48,XXXX were also seen, but the proportion of abnormal X chromosome was very low. Short stature was observed in deletions of Xp.

C-17

DE NOVO t(2;15) TRANSLOCATION IN A BOY WITH PROBABLE SHPRINTZEN-GOLDBERG SYNDROME. Shinsuke NINOMIYA, Ryozo KASAI (Asahigawa Jidoin Children's Hospital, Okayama), Koji NARAHARA, Yuji YOKOYAMA, and Yoshiki SEINO (Dept. Pediatr. Okayama Medical School, Okayama)

Shprintzen-Goldberg syndrome (SGS) represents a clinical syndrome characterized by craniosynostosis and mental retardation in addition to Marfan syndrome findings. Recently, mutation of FBN1 were detected in two patients with this syndrome. We reported a 10-year-old mentally retarded boy who had characteristic craniofacial dysmorphism, arachnodactyly, retentio testis, and tetralogy of Fallot. His phenotype is consistent with SGS. Chromosomal analysis showed a karyotype of 46,XY,t(2;15)(q24.2;q21.2) or (q31.1;q22.1) de novo. The localization of the 15q breakpoint in this patient at 15q21 to which FBN1 is mapped and the report of mutation suggest that FBN1 may be a candidate gene of SGS.

C-18

ANALYSIS ON A RELATIONSHIP BETWEEN THE PHENOTYPE AND FREQUENCY OF TRISOMIC CELLS IN FIVE MOSAICS FOR TRISOMY 20

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Approximately 90% of trisomy-20 mosaics were associated with a grossly normal phenotype, and 20-trisomic cells have not been detected in their blood cultures. It is likely that, in the majority of cases, cells with trisomy 20 were extraembryonic in origin or largely confined to certain fetal organs. Moreover, it is impossible to determine the tissue origin of 20-trisomic cells in amniocytes. Thus, trisomy-20 mosaicism has a serious dilemma in prenatal diagnosis and genetic counseling. We experienced 5 cases of trisomy 20 mosaicism among 4,782 cases with prenatal chromosomal analysis, and examined whether the phenotype is related to the frequency of trisomic cells in these mosaics. Of 4 cases whose trisomic cell-frequency was less than 60%, two showed a normal phenotype but the other two were uninformative. One case with 94% of cell frequency showed a slightly abnormal phenotype without renal and heart abnormalities typical for reported trisomy-20 mosaics, indicating that a high mosaic frequency is not always related to severe abnormalities.

C-19

A 12q PARTIAL MONOSOMY(q11-13.1) BOY WITH A COMPLEX TRANSLOCATION INVOLVING CHROMOSOME 12, 13, AND 21. Mitsuyo SASAKI (Dept. Pediat., Wakayama Medical College, Wakayama), Ryuichi TSUKINO (Dept. Pediat., Arida City Hospital, Wakayama), Kiichi SHIMIZU, and Takahiro MINESHITA (Shionogi Biomedical Laboratories, Osaka)

We report on a 4-year-old boy with mild mental retardation and an apparently unbalanced de novo translocation involving chromosomes 12, 13, and 21, and six break points (4 points in chromosome 12, one point in 13 and 21, respectively). To determine detail break points, whole painting probe and high resolution G-banding was complexed, and he was diagnosed 12q partial monosomy due to interstitial deletion 12q from q11 to q13.1

The karyotype was 46,XY,+der(12)t(12;13;21)(21pter→21q21::21p21.2→12q11::12q21.2→12q13.1::13q32→13pter), +der(13)t(12;13)(q21.2;q32), +der(21)t(12;21)(p12.2;q21).

In our knowledge, this type deletion was not found.

The child had a hirsutism, long eyelashes, strabismus, synophrys, flat nasal bridge, bulbous nose, arched mouth, short philtrum, full cheeks, low hair line, simian lines, moderate short stature (-1.5SD) and mild mental retardation.

C-20

A Yq- CASE OF SHORT STATURE WITH Q-BANDING NEGATIVE HETEROCHROMATIN REGION. Kyoung Chang KIM, Kazunari MATSUSHIMA, Tsutomu OHNO, Hirofumi OHASHI (Saitama Children's Med. Ctr, Saitama) and Keiko WAKUI and Yoshimitsu FUKUSHIMA (Shinshu Univ. Sch. Med., Nagano)

The patient, a male, was born at 39 weeks of gestation to healthy and unrelated parents. His birth weight was 1,440 g (-4.4 SD) and length 40 cm (-5.3 SD). G-banded chromosome analysis revealed a 46,X,+mar karyotype. By FISH with Y specific centromeric repetitive probe (DYZ3), the marker was found to originate from Y. We diagnosed that it was Yq-. Q-banding showed no fluorescent heterochromatin region on the Yq, though other cytogenetic and FISH analyses revealed a tiny but obvious heterochromatin segment at its end. At 14 months his length was 71 cm (-2.3 SD) and his development was normal. His father had a normal Y chromosome. The break of the Yq in this patient seems to separate the heterochromatin region of Yq into Q-banding positive and negative segments. Furthermore, this case may suggest that putative growth control genes exist at this Q-banding negative heterochromatin segment of Yq.

C-21

Monozygotic twins with CATCH22 and discordant phenotype
Hiroyuki YAMAGISHI, Chihiro ISHII, Jun MAEDA, Yoshifumi KOJIMA, Nobutake MATSUO (Dept. Pediatr., Keio Univ., Tokyo), Rumiko MATSUOKA, Misa KIMURA, Atsuyoshi TAKAO, Kazuo MOMMA (Tokyo Women's Medical College, Tokyo)

CATCH22 (cardiac defect, anomaly facies, thymic hypoplasia, cleft palate and hypocalcaemia) is due to deletion on chromosome 22q11. Goodship et al. recently described 22q11 deletion in monozygotic twins having discordant phenotypes (J. Med. Genet. 1995; 32: 746-8). We report on male monozygotic twins with CATCH22 in whom phenotypic discordance was more conspicuous. They were delivered at 37 weeks' gestation with twin 1 weighing 1572g and twin 2 weighing 2376g. Twin-twin transfusion syndrome was diagnosed (hemoglobin, twin 1; 10.4 g/dl vs twin 2; 17.4 g/dl). FISH analysis showed microdeletion on chromosome 22q11 in each and various blood types were identical (probability of monozygosity of 99.95%). Twin 1 had cardiac defect, characteristic facial appearance, swallowing dysfunction, anal atresia, and mental retardation. Twin 2 had characteristic facial appearance but no other stigmata of CATCH22. Our observation further suggests that phenotypic variability in CATCH22 may be in part due to early intrauterine environmental factors. The role of twin-twin transfusion syndrome appears to be subsidiary, since it occurs late in intrauterine life.

C-22

Evolving clinical spectrum in CATCH22 : Observations on 20 patients
Jun MAEDA, Hiroyuki YAMAGISHI, Rika KIZU, Yoshifumi KOJIMA, Nobutake MATSUO (Dept. Pediatr., Keio Univ., Tokyo)

Chromosome 22q11 deletion syndrome, CATCH22 (cardiac defect, anomaly facies, thymic hypoplasia, cleft palate and hypocalcemia) is known to present a wide clinical spectrum. We report on subtle features of velo-pharyngeal insufficiency and hypoparathyroidism in 20 patients (14 boys and 6 girls, ages 1 - 15 years) of CATCH22. Abnormalities found in these patients included anomaly facies in 20, cardiac defects in 16, recurrent aspiration pneumonia in 9, velo-pharyngeal insufficiency in 12, and hypocalcemia in 10. Of the 12 patients with velo-pharyngeal insufficiency, one had overt cleft palate that was diagnosed during neonatal period, and five had occult (submucous) cleft palate that was diagnosed during late infancy when assessment of speech became feasible. Of the 10 patients with hypocalcemia, four had overt hypoparathyroidism manifested by neonatal tetany or convulsion, and six had latent hypoparathyroidism with no clinical sign and symptom. These six patients developed overt hypocalcemia in infancy after prolonged fasting, and two of the six developed overt hypoparathyroidism in puberty. These observations indicate that the clinical definition of CATCH22 awaits a careful follow-up of similarly affected patients from early infancy to adulthood.

C-23

CORRELATION BETWEEN SMALLER DELETION SIZE AND MENTAL
RETARDATION IN del22q11.2 SYNDROME.

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DiGeorge anomaly (DGA), conotruncal anomaly face syndrome (CAFS), velo-cardio-facial syndrome and Opitz GBBB syndrome have similar but variable phenotypes, and share the deletion of 22q11.2 as a common feature. One hundred and seventy seven CAFS and 18 DGA patients, who had del22q11.2 detected by fluorescent *in situ* hybridization (FISH) using a N25 (D22S75) DGCR probe, were examined to compare deletion size and phenotypes. Five out of 177 patients had smaller deletion sizes than 2 Mbp and 3 of them had no mental retardation. CAFS and DGA without CAF patients who did not have del22q11.2 were examined by FISH using a SD10p1 probe. No deletion of 10p region was detected.

C-24

IDENTICAL TWINS DISCORDANT FOR TURNER SYNDROME: FISH ANALYSIS USING INTERPHASE NUCLEI OF BUCCAL MUCOSA. Keiko WAKUI¹, Makoto MIKAWA², Yoshimitsu FUKUSHIMA¹ (Dept. of Hygiene and Medical Genetics, Shinshu University School of Medicine, Matsumoto¹, Kitami Red-Cross Hospital, Kitami²)

Conventional chromosome analysis using peripheral lymphocytes revealed mos 45,X/46,XX in both 16-month-old identical twins. The frequency of 45,X cell was 5/30 (17%) in Twin 1 and 8/30 (27%) in Twin 2. Twin 2 had typical features of Turner syndrome including short stature, webbed neck and peculiar facies, while Twin 1 was phenotypically normal. Detection of the number of chromosome X was performed on buccal smear preparations from the twins by FISH using DXZ1 probe. 500 nuclei were observed in each case. The frequencies of each number of signals of chromosome X [1:2:3 (signals)] were: [3:4: 95.8: 0.8 (%)] in Twin 1, [96.7: 3.3: 0 (%)] in Twin 2 and [2.3: 95.4: 2.3 (%)] in normal female control. These findings suggest that loss of an X chromosome occurred early in embryogenesis with complete separation of 45,X and 46,XX cell lineages at the first cleavage division of the zygote, and the mosaicism in the peripheral blood of the twins is due to twin-to-twin transfusion resulting in a mixture of the two-cell populations in the hematopoietic tissue. Interphase FISH on buccal smear preparations is a rapid, effective and non-invasive method for studies of mosaicism.

C-25

DETECTION OF ADDITIONAL CRYPTIC CHROMOSOME REARRANGEMENTS BY FISH IN PATIENTS WITH STRUCTURAL CHROMOSOME ABNORMALITIES: REPORT OF TWO CASES. Kazushige MATSUSHIMA, Keiko WAKUI, Takeshi ITO, Kyoung Chang KIM(Saitama Children's Medical Center), Shoko TSUNODA (Umeda Akebono Clinic, Tokyo), Masato TSUKAHARA (Yamaguchi University, Yamaguchi), Yoshimitsu FUKUSHIMA (Shinshu Univ. Sch. Med., Nagano), Hirofumi OHASHI (Saitama Children's Medical Center, Saitama)

We report on two patients with structural chromosomal abnormalities who have additional cryptic chromosome rearrangements unidentifiable on standard chromosome analyses. Case 1: The patient was a 12-year-old girl with features of mental retardation, characteristic facies and short stature. G-banding chromosome analysis showed that she had a direct duplication of 4q31.3->q35. FISH with 4q distal region painting probe confirmed this duplication, and further revealed additional cryptic translocation between the duplicated 4q region and chromosome 1 in 83% cells examined. Case 2: The patient was a 10/12-year-old boy with features of developmental delay, infantile spasms and linear skin pigmentation. G-banding analysis showed that he had a marker chromosome in 82% cells examined. The marker was diagnosed as inv dup(15) by DA/DAPI staining. FISH with D15Z1 (alpha-satellite probe specific for chromosome 15) confirmed this diagnosis, and further detected three D-group chromosomes with a D15Z1 signal. Q-banding -> FISH with D15Z1 sequential analysis indicated that the extra D-group chromosome was chromosome 14 whose centromeric region was replaced by that of chromosome 15.

C-26

AN ACCURATE GENDER DETERMINATION ASSAY FROM A SMALL AMOUNT OF DNA SPECIMEN AS AN AID FOR NON-INVASIVE PRENATAL DIAGNOSIS.

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For clinical application of genetic diagnosis from blastomeres, exfoliative fetal cells of the uterine cervix, or circulating fetal cells in the maternal blood, we have developed an accurate multiplex polymerase chain reaction (PCR) assay for gender determination from a small amount of DNA specimen. This assay co-amplifies X- and Y-specific repetitive sequences (DXZ1/DYZ1), so that 2 bands (DXZ1 and DYZ1 positive) implies male; single band (only DXZ1) means female; no band suggests amplification failure or sampling error. Each band was confirmed to be specific by the direct PCR products sequencing method. To evaluate accuracy and efficiency of this assay in single cell, we sampled a single amniocyte (n = 50) with a micromanipulator under an inverted microscope, extracted DNA from it, and analyzed. Sex determination by this PCR method was compared with that by karyotyping. The amplification success rate was 98% (49/50) and misdiagnosis was not observed. This newly developed assay appears efficient and accurate, thus may be applicable to the above mentioned clinical settings.

C-27

APPLICATION FOR PRENATAL DIAGNOSIS OF FLUORESCENCE IN SITU HYBRIDIZATION METHOD - ANALYSIS OF CHROMOSOMAL STRUCTURAL ABNORMALITIES - Nobuhiro SUZUMORI, Mitsuyo TANEMURA, Kaoru SUZUMORI (Dept. Obstet. Gynecol. Nagoya City Univ. Hospital, Nagoya), Keiei KIM, Hirofumi OHASHI (Dept. Genetics. Saitama Children's Medical Centre, Iwatsuki City, Saitama) and Keiko WAKUI, Yoshimitsu FUKUSHIMA (Dept. Public Health, Shinshu Univ., Matsumoto)

We studied that, for two pregnant women with chromosomal structural abnormalities, analysis of chorionic villi cells and amniotic fluid cells culture was available or not, as prenatal diagnosis. Case1; 34-year-old-pregnant woman has had three times spontaneous abortions and her chromosome is 46, XX, ins(1;2)(q36;q22q24). Case2; 31-year-old-pregnant woman's chromosome is 46, XX, t(3;12)(p26.2;p13.1) and her first kid had chromosomal abnormalities. Therefore, after informed consent, prenatal diagnosis was occurred by fluorescence in situ hybridization (FISH) method. Case1 (from chorionic villi); 2a-satellite probe, cosmid probe cCI 2-603, Case2 (from amniotic fluid); 12a-satellite probe, 12 whole painting probe, 3p-distal painting probe, cosmid probe cCI 12-134. After the FISH analysis, Case1; fetal chromosome was same to the mother. Case2; fetal chromosome was 46, der(3)(3;12)(p26.2;p13.1). We admitted that FISH method was available for prenatal diagnosis of a patient, who had chromosomal structural abnormalities.

C-28

IS THERE ANY PREDICTIVE VALUE IN CHROMOSOME ANALYSIS OF SPONTANEOUS ABORTUSES FOR THE OUTCOME OF SUCCEEDING PREGNANCIES? Kodo SATO, Tomoyuki MIYAKAWA, Kyoko SHIODA (Dept. Obstet. Gynecol., Toranomon Hospital, Tokyo), Junko IMAMURA and Ryou NAKASONE (Dept. Hematol., Toranomon Hospital, Tokyo)

Predictive value of chromosome analysis of spontaneous abortuses for the outcome of succeeding pregnancies were studied, using 281 spontaneous abortions (1st AB) where chromosome analysis was carried out and another pregnancies (2nd PREG) were achieved after the 1st AB. The maternal age at the 1st AB and the incidence of spontaneous abortion among the 2nd PREG was revealed to be significantly correlated ($p < 0.01$), and the following formula was calculated. $Y = 0.00433X^2 - 0.267X + 4.156$ (Y: incidence of abortion among the 2nd PREG, X: maternal age at the 1st AB). Thus, the expected incidence of abortion among the 2nd PREG was calculated. In cases with normal karyotype for the 1st AB, the incidence of spontaneous abortion among the 2nd PREG was 7/75, whereas the expected incidence was 9.1/75, thus the ratio of the real incidence against the expected was 0.77; in trisomy (121 cases) the ratio was 0.87; in triploidy (17 cases) 2.28; in tetraploidy (8 cases) 2.52; in 45,XO (23 cases) 0.78; in mosaicism (27 cases) 0.95, in structural abnormalities (6 cases) 0.00; in other abnormalities (4 cases) 4.76. No significant difference was observed for ratio of the real/expected incidence between the different chromosome abnormalities. Thus the chromosome analysis of the spontaneous abortuses was revealed to have of no or little predictive value for the outcome of the following pregnancies.

C-29

PRENATAL DIAGNOSIS OF WILLIAMS SYNDROME BY FISH. Mashio KITATANI, Mamoru OZAKI, Hiroaki TAKAHASHI (Clin. Genet. Kanazawa Med. Univ.), Tomoko INOUE (Toyonaka City Hospital), Toshikatu NOBUNAGA (Osaka Med. Center & Res. Inst. for Maternal & Child), Fumitaka SAJI, Yuji MURATA (Osaka Univ. Med. Sch.)

The Williams syndrome (WS) is caused by hemizyosity of elastin gene (ELN) on a chromosome region 7q11.23. A monosomy of an elastin locus can not be detected by cytogenetically except for a few cases associated with chromosomal rearrangement. Fluorescent in situ hybridization (FISH), using the Williams Syndrome Chromosome Region probe, has been shown to be useful for the diagnosis of WS in lymphoblast. We performed a prenatal diagnosis at the request of the parents whose first child had been a Williams syndrome. Amniotic fluid cells obtained at 15 weeks of gestation, cytogenetical analysis revealed 46,XX. Hemizyosity for a region of 7q11.23, involving the elastin locus, was found in the first child, but was not found in the amniotic fluid cells of the fetus by FISH. The Pregnancy proceeded and resulted in a healthy baby.

C-30

A CASE OF PARTIAL TRISOMY 11p DERIVED FROM MATERNAL BALANCED TRANSLOCATION. Tomoyuki MIYAKAWA, Kyoko SHIODA, Kodo SATO (Dept. Obstet. Gynecol., Toranomon Hospital, Tokyo)

Partial trisomy 11p is rare chromosomal abnormalities and was first described by Francke(1972) and Falk et al.(1973), and approximately 20 cases have been reported. Trisomy 11p was detected by amniocentesis on 24-year-old, gravida 0 woman who was pointed out oligohydramnios and intrauterine growth retardation(IUGR) by ultrasonography at 27 weeks gestation. The result of amniocentesis was 46,XY,der(14)t(11;14). Chromosomal analysis of the parents was performed and their karyotypes were 46,XX,t(11;14)(p15.3;q32.3), respectively. At 29 weeks gestation, rupture of the membrane occurred and a 572 gm stillborn male fetus was delivered at 33 weeks gestation. The baby had prominent forehead, flat nasal bridge, large nose, hypertelorism, low-set ears, cryptorchidism, and hypospadias. Macroglossia and other major anomalies were not recognized. Oligohydramnios and severe IUGR are rare abnormality not previously described in partial trisomy 11p.

C-31

PRENATAL DIAGNOSIS OF LOW FREQUENCY MOSAICISM FOR SUPERNUMERARY INV DUP(15). Kiyoko KOYAMA, Koji NARAHARA, Yuji YOKOYAMA, Michio TERAOKA, Yoshiki SEINO (Dept. Pediatr. Okayama Univ. Okayama), Katsuhiko TADA and Takafumi KUDO (Dept. Obstet. Gynecol.)

The presence of mosaicism for chromosome imbalance imposes a great challenge to prenatal diagnosis. Although a half of all supernumerary marker chromosomes are derived from a chromosome 15, inv dup(15)s have rarely been detected in prenatal diagnosis. We report on a case of low frequency mosaicism for supernumerary inv dup(15). A couple underwent prenatal diagnosis because of advanced maternal age. The first amniocentesis showed that a small proportion of cells (18/83) had an extra G-sized chromosome. The second amniocentesis and cordocentesis also showed the same abnormality in a minority of cells. The interrupted pregnancy resulted in an apparently normal female fetus with no malformations of internal organs. FISH studies using D15Z1 and GABRB3 probes confirmed that the extra chromosome is an inv dup(15). This case illustrates the importance of repeated examinations of different tissues in confirming the low frequency mosaicism as well as the need of molecular tests for predicting the clinical outcome of patients with inv dup(15)s detected prenatally.

C-32

ORIGIN AND MECHANISM OF MIXOPLIIDY COMPOSED OF 45,X AND 47,XX,+21 CELL LINES IN A FETUS

Naoki HARADA, Kyohko ABE, Tomoko NISHIMURA, Masakazu SOUDA, Kazuo NODA (Kyushu Med. Sci., Nagasaki), Kiminori SASAKI, Mutsuo ISHIKAWA (Dept. Obstet. Gynecol., Asahikawa Med. Coll., Asahikawa), Tadashi MATSUMOTO and Norio NIKAWA (Dept. Pediat. & Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Chromosome analysis of amniotic fluid cells from a 17-week-old fetus suffering from cystic hygroma colli revealed a mixoploid composed of 45,X and 47,XX,+21 karyotypes. Each cell line was also observed in other tissues including skin, cord blood, amnion and chorionic villus tissues of the fetus, which were collected after termination. We studied the origin and the mechanism of formation of this unique mixoploid using chromosomal heteromorphisms and DNA polymorphisms as genetic markers. All the heteromorphic markers examined showed no discordance in parents-to-child transmission or between the two cell lines. DNAs were extracted from monoclonal cells with each karyotype established from the fetus skin fibroblasts using the limited dilution method. These DNAs and those extracted from peripheral blood leukocytes of the parents were analyzed with 8 polymorphic markers, including one RFLP and 7 VNTRs. Three VNTR markers were informative in this analysis and the two cell lines had identical alleles transmitted from the parents. While the other 4 VNTRs and 1 RFLP markers were uninformative, because both parents were homozygous for these loci. From these findings, we concluded that a zygote had either a 45,X or a 47,XX,+21 karyotypes, and then subsequent loss of X chromosome or gain of chromosome 21 due to anaphase lag or somatic nondisjunction, leading to the mosaicism.

C-33

CHARACTERIZATION OF ISOCHROMOSOME 18q IN PRENATAL DIAGNOSIS

BY G-BANDING METHOD AND FLUORESCENCE IN SITU HYBRIDIZATION.
Satoshi HAYSHI, Maki HYODO, Masayuki KINUTANI, Hisaya FUJIWARA, Kenjiro DATE, Tomoya MIZUNOE, Yoshinori KISAKA, Takeshi URABE (Dept. Obstet. Gynecol., Hiroshima Prefectural Hospital, Hiroshima) and Norio MIHARU, Koso OHAMA (Dept. Obstet. Gynecol., Hiroshima Univ. Sch. of Medicine, Hiroshima)

The patient was referred for cytogenetic analysis because of polyhydramnios and abnormal findings of the fetus by ultrasonography at a gestation age of 28 weeks. She was a 29-year-old primigravida. Cordcentesis was performed at 29 weeks' gestation. Cytogenetic evaluation from cord blood showed 46, XX, -18, +i(18q) in 20 metaphases by G-banding method. And fluorescence in situ hybridization (FISH) was performed to certify isochromosome 18q using alphasatellite probe specific for chromosome 18 (Oncor Inc., Gaithersburg, MD). Two strong signals were observed, confirming an isochromosome 18 replacing one normal chromosome 18. The baby was stillborn at a gestation age of 30 weeks. The baby's weight was 1150g. At pathological examination, there were short neck, low-set ears and small round nose. The cerebral ventricle was dilated. The left diaphragmatic hernia was observed and the left lung was atrophic. The right lung had two lobes and there was pleural effusion. Intracardinally ventricular septal defects (9×10mm) was found. The umbilical hernia and imperforate anus were found. The characteristic features of this baby were similar to 18 trisomy syndrome but did not present features of 18pmonosomy syndrome.

C-34

MONOZYGOTIC TWINS CONCORDANT OR DISCORDANT FOR MALFORMATIONS OF MUSCULO-SKELETAL SYSTEM. Yasuo SUGIURA, Tsuyoshi UEKE (Kanayama Clinic, Nagoya) and (Chubu Rehabilitation College, Nagoya)

Since 1954, 6 monozygotic twins have been observed at Nagoya University Hospital, Nagoya City University Hospital and their affiliated hospitals. Diagnosis of monozygosity was decided with various types of gene markers.

Concordant twins were 1 pair for achondroplasia, 1 pair for symphalangism and 2 pairs for postaxial polydactyly of toes. Discordant twins were 1 pair for arthrogryposis multiplex congenita and 1 pair for congenital unilateral short femur.

Concordant twins were considered to be caused from genuine genetic factor, while discordant twins were considered to be caused from interaction between genetical and fetal environmental factor.

C-35

ULTRASOUND SCREENING OF NEWBORN INFANTS FOR KIDNEY AND URINARY TRACT ABNORMALITIES; REPORT OF 14 NEONATES WITH CONGENITAL HYDRONEPHROSIS. Akira MATSUI*, Tsunehisa TAKENAKA** (Dept. Pediat*, and Obstet.Gynecol** Iseaki Municipal Hospital, Gunma) and Masamichi HAYASHI, Hidetoshi YAMANAKA(Dept. Urol., Gunma University School of Medicine, Gunma)

For the purpose of early detection and early treatment of kidney and urinary tract abnormalities, ultrasound screening of newborn infants has been done. A total of 4,610 newborn infants, born at Iseaki Municipal Hospital from October 1988 to October 1996 were examined by ultrasound. Most of them were scanned between 2 to 7 days after birth, using Aloka SSD 650 scanning system equipped with a 5-MHz linear transducer. When abnormal scans were found serial ultrasound studies, urinalyses and uro-radiologic examinations were carried out. Out of 4,610 newborn infants 14 revealed congenital hydronephrosis. In this paper, clinical and laboratory findings of these 14 cases(12 males and 2 females) were presented. All these cases showed unilateral hydronephrosis(right kidney in 5 and left in 9). By ultrasound findings at least one side of the renal pelvis was found to be dilated. Grade 2 hydronephrosis was found in 6 cases, grade 3 in 3 and grade 4 in 5. Surgical treatment was applied in 5 cases. Another 5 cases were followed nonoperatively and 4 cases were lost to follow-up.

In conclusion ultrasound may be useful for the screening of congenital hydronephrosis at neonatal period.

C-36

A SECOND CASE OF KABUKI-MAKE-UP SYNDROME WITH LOWER PITS.
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The girl was the first child of a 29-year-old mother and a 33-years old father. She had soft cleft plate and lower lip pits. The diagnosis of Van der Woude syndrome (VWS) was made. She was first evaluated by us at the age of 3 years because of her short stature. She had postnatal onset growth retardation, typical facial appearance, typical dermatoglyphic pattern, partial growth hormone deficiency and a low normal limit mentality. She was then also diagnosed as having Kabuki make-up syndrome (KMS).

The girl was the second case, who represented 2 independent dominant syndromes, KMS and VWS. Recently, gene locus of VWS-1 is assigned at 1q32-41, by microsatellite-based mapping [Sanger et al., 1995]. But her high resolution chromosome failed to show any deletion and aberration on this region.

C-38

FAMILIAL PORO-CYSTIC OSTEODYSPLASIA WITH TARDY PROGRESSIVE PRESENILE DEMENTIA. Isao SUGIURA (Dept. Orthop. Surg., SL Medical Centr, Nagoya), Yasuo SUGIURA (Kanayama Clinic, Nagoya) and Toshiyuki FURUSHO (Dept. Hum. Genet., Kyohrin Univ., Tokyo)

This disease is characterized by skeletal manifestations with multiple poro-cystic lesions associated with pathologic fractures and by neuropsychiatric dysfunctions with progressive dementia. CT of brain shows marked signs of cortical atrophy and dilatation of the ventricles. The intramedullary lesions are filled with a molted cheese-like adipose tissue which shows histopathologically peculiar characteristic undulating membrano-cystic structures, reacting to PAS, Luxol fast blue and Sudan III. Electron-microscopically, membrano-cystic lesions are composed of numerous minute tubular structures arranged perpendicularly to the inner surface.

The authors carried out an investigation of 91 Japanese patients of 79 families including their 7 own families with special emphasis on genetical analysis. As regard to consanguinous marriage of the parents, first cousins was found in 34 families, no consanguinity in 22, unknown in 16 and other consanguinity than first cousins in 7. The inheritance of this disease strongly suggests an autosomal recessive, and recessive gene frequency calculated by Dahlberg's formula showed extremely low : $q = 0.00038$ (1972-1977) and $q = 0.00009$ (1978-1983).

C-39

A JAPANESE PATIENT OF RESTRICTIVE DERMOPATHY. Mihoko NAKAMURA(Dept. Pediatr., Kagoshima University School of Medicine, Kagoshima), Kiyoshi IMAIZUMI, Takuma ISHII, Mitsuo MASUNO, Yoshikazu KUROKI(Div. of Med. Genet., Kanagawa Children's Med. Ctr., Yokohama), Gen NISHIMURA(Dept. of Radiol., Dokkyo Univ. School of Med., Tochigi)

Restrictive dermopathy is a rare lethal autosomal recessive skin dysplasia with multiple congenital contractures and characteristic facies. We report a girl, the first child of healthy and non-consanguineous Japanese parents. Intrauterine growth retardation of the baby, pulmonary hypoplasia, fixed flexion contractures, and decreased movement with polyhydramnios were revealed on a ultrasound at 31 weeks gestation. High echogenic lesion of each orbit were also observed, which were turned out to be the ectropion of edematous eyelids after birth. She was born at 32 weeks gestation, birth weight was 958g, OFC 27cm. Clinical findings included a tight, thin, translucent skin, round face with hypertelorism, edematous and ectropion of eyelids, small pinched nose, low-set ears, small fixed open mouth, protuberant tongue, and micrognathia. Multiple joint contractures and rocker-bottom feet were also observed. She died shortly after birth. Radiographs showed large anterior fontanel, thin and irregular ribs, and hypoplastic clavicles. The ulnar shafts were particularly narrow distally as were the radial shafts proximally. Radiological findings were useful for the diagnosis of Restrictive dermopathy.

C-40

FAMILIAL CASE OF Holt-Oram SYNDROME AND VACTERL ASSOCIATION. Takuma ISHII¹, Mitsuo MASUNO¹, Kiyoshi IMAIZUMI¹, Yoshikazu KUROKI¹, Hiroshi TAKE², Toshiji NISHI² (¹Div. of Medical Genetics, ²Dept. of Surgery, Kanagawa Children's Medical Center, Yokohama, Japan)

We report on a first familial case of Holt-Oram syndrome and VACTERL association. Case1 is a 3-month-old boy with VACTERL association. He is diagnosed by agenesis of sacrum, anal atresia, atrial septal defect, patent ductus arteriosus, cystorectal fistula, hypospadias, bifid scrotum and limb anomalies (right side is dominant). Case2 is a 21-years-old female with Holt-Oram syndrome. She is a mother of Case1. She is diagnosed by limb anomalies (left side is dominant), ventricular septal defect, patent ductus arteriosus and mitral valve insufficiency. To explain this case, we make three hypotheses. (1)A part (or all) of VACTERL association is the same disease as Holt-Oram syndrome. (2)A part (or all) of VACTERL association and isolated type Holt-Oram syndrome are the same single-gene inheritance disease. (3)A part (or all) of VACTERL association is a contiguous gene syndrome including Holt-Oram syndrome.

To be clear the relation between Holt-Oram syndrome and VACTERL association, description of additional cases and linkage analysis are necessary.

C-41

JAPANESE GIRL WITH NAGER SYNDROME (PREAXIAL ACROFACIAL DYSOSTOSIS)
Satoshi ISHIKIRIYAMA, Tamaki NUMAZAWA, Tsuneho NAKAMURA, Fumiyo KUDOH
(Chiba Children's Hospital, Chiba) and Michiyo EBARA (Chiba Rehabilitation center, Chiba)

A female baby was born to healthy, non-consanguineous parents after an uneventful pregnancy. At birth, her father was 34 years old, her mother 29 years old, respectively. Both of the parents were Brazilian descended from Japanese. She was an only child of the parents. Her birth weight was 3,276 g (+4.4 SD), height 48.0 cm (-0.47 SD), respectively. She had not only downward slanted palpebral fissures, depressed nasal bridge, and severe micrognathia but also bilateral hypoplastic thumbs. No other family members had micrognathia and hypoplastic thumbs. She suffered from respiratory distress and failure to thrive during neonatal period. She needed nasal tubing during her infancy. Her karyotype was normal female: 46,XX.

Nager syndrome is genetically heterogeneous, partly autosomal dominant and partly autosomal recessive in inheritance, while Treacher-Collins syndrome is completely autosomal dominant. It is important to distinguish Nager syndrome from Treacher-Collins syndrome, so also necessary to try to detect radial anomalies when one examines a patient with micrognathia.

C-42

JAPANESE PATIENTS WITH X-LINKED α -THALASSEMIA/MENTAL RETARDATION SYNDROME. Kenji KUROSAWA, Akira AKATSUKA, Yukikatsu OCHIAI, Masako ASOH, Jun IKEDA, (Tokyo Metr. Kita Med. Rehabil. Ctr. for The Handicapped, Tokyo) and Kihei MEKAWA (Dep. Pediatr., Jikei Univ. School of Medicine, Tokyo)

X-linked α -thalassemia/mental retardation syndrome (ATR-X) is characterized by severe mental retardation, wide range of minor abnormalities, and association with an unusual form of α -thalassemia. We report two Japanese patients with ATR-X. In addition to their dysmorphic features including a triangular nose with upturned nares, a broad nasal bridge, nasal alae extending lower than the columella and septum, carp-shaped mouth with full lower lip, and widely spaced teeth, incubation of their blood with 1% BCB showed 0.1-0.4% red cells with HbH inclusions. Both cases are sporadic ones. In the case of the severely mentally retarded, it is important to take this disorder into consideration for differential diagnosis. Other dysmorphic mental retardation, which are linked to the Xq12-q21 region, could be due to mutations in XH2/XNP.

C-43

POPULATION SCREENING OF FRAXE: CYTOGENETIC AND PCR ANALYSES IN MALES WITH SEVERE MENTAL RETARDATION. Ryozo KASAI¹, Koji NARAHARA², Shinsuke NINOMIYA¹, Yuji YOKOYAMA², Kazushiro TSUJI² and Yoshiki SEINO² (¹Asahigawa Jidoin Child. Hosp., Okayama and ²Dept. Pediatr., Okayama Univ., Okayama)

Expansion of the (CCG)_n triplet repeat at the FRAXE is associated with mental impairment in some individuals. To determine the frequency of FRAXE repeat expansion among persons with severe or profound mental retardation, institutionalized 103 males (age of 11 to 66-years-old) were studied by using cytogenetic and molecular method. Cytogenetic studies revealed that 4 out of 103 individuals showed fragile sites at Xq27-28 region between 2 to 6 % of observed cells. It seems likely that four cases with low incidence of fra(X)(q27-28) expression have a common fragile site in this region. On the other hand, FRAXE PCR analyses using primers 598 and 603 (Knight et al., 1993) revealed that all of 103 individuals had PCR products of 300 to 350 bp length which correspond to 4 to 21 (CCG)_n normal repeats size. These results suggest that FRAXE (CCG)_n repeat expansion is not a cause of severe or profound mental retardation in insititutionalized developmentally delayed males .

C-44

SMITH-LEMLI-OPITZ SYNDROME IN JAPAN
Masato TSUKAHARA, Keiko FUJISAWA, Kiyomi YAMAMOTO
(School of Allied Health Sciences, Yamaguchi Univ., Ube)

Smith-Lemli-Opitz (SLO) syndrome is caused by inborn error of cholesterol synthesis. The syndrome is characterized by multiple congenital anomalies/mental retardation, including characteristic facial appearance, microcephaly, growth retardation, anomalies of the limbs, and genitalia. The major biochemical abnormalities in the SLO syndrome consist in reduced serum cholesterol associated with increased 7-dehydrocholesterol concentrations. We sent questionnaire to 554 hospitals to estimate the number of the patients with the SLO syndrome, including University hospitals that were registered by Japan Society of Pediatrics. The response rate was 419/554 (75.6%). A total of 46 individuals from 24 hospitals were reported to have the SLO syndrome. Of these, one patient was biochemically diagnosis as having the SLO syndrome, 25 were diagnosed on the basis of clinical manifestations, and 20 were suspicious of having the SLO syndrome. The occurrence in monozygotic twins was reported in one family. Eight patients were died, and 38 are still alive. The serum 7-dehydrocholesterol concentrations were determined in 16 patients. All the 16 patients with the suspicion of having the SLO syndrome had normal levels of the serum 7-dehydrocholesterol. Thus, definite diagnosis was made in one case, and the SLO syndrome was biochemically denied in 16 patients.

The diagnosis of the SLO syndrome seems ambiguous because of its wide spectrum of multiorgan involvement. The serum 7-dehydrocholesterol determination combined with clinical manifestations is necessary to make a definite diagnosis of the SLO syndrome.

C-45

A NEW CASE WITH SHPRINTZEN-GOLDBERG SYNDROME WITH BILATERAL ECTOPIA LENTIS. Yoriko WATANABE, Shoji YANO, Yoko SUGAHARA, Yasutoshi KOGA, Makoto YOSHINO, Hirohisa KATO (Dept. Pediat. Kurume University School of Medicine, Kurume)

Shprintzen-Goldberg syndrome (SGS) is a rare disorder with characteristic features including Marfanoid habitus, arachnodactyly, camptodactyly, craniosynostosis, exophthalmos, maxillary and mandibular hypoplasia, palatal shelf soft tissue hypertrophy, infantile hypotonia, and mental retardation. SGS was thought to be a generalized connective tissue dysplasia until Dietz et al. recently reported that SGS is associated with mutations in the same gene that is involved in Marfan syndrome, i.e. fibrillin-1 (FBN-1). We experienced a 9 year-old Japanese boy with clinical phenotype of SGS with bilateral ectopia lentis. He had generalized hypotonia, developmental delay, gingival hypertrophy, and developed dilatation of the aortic root at 3 years of age. He was initially diagnosed with Marfan syndrome because of the mild craniofacial involvement with mild proptosis, strabismus, and facial asymmetry. Ectopia lentis is not a typical finding in SGS although it is known as one of cardinal manifestations in Marfan syndrome. Molecular and functional analysis of FBN-1 gene and its product in this case may help to understand the molecular basis of SGS involving central nervous and craniofacial skeletal system which are not associated with Marfan syndrome.

C-46

IDENTICAL TWINS DISCORDANT FOR COHEN SYNDROME. Hirofumi OHASHI, Kyoung Chang KIM, Shinichiro HAMANO, Masato SATO, Kibo YOSHIDA (Saitama Children's Medical Center, Saitama), Fumie TAKAKUBO, Ikuko KONDO (Ehime Univ. Sch. Med., Ehime), Yoshimitsu FUKUSHIMA (Shinshu Univ. Sch. Med., Nagano)

Cohen syndrome is a congenital malformation syndrome which follows an autosomal recessive mode of inheritance. The gene locus responsible for Cohen syndrome is assigned around 8q22. We report on a pair of female identical twins discordant for Cohen syndrome. The twins were born at 36 weeks' gestation to unrelated parents. The patient's twin sister and her elder brother were normal. The features of the patient were mental retardation, hypotonia in infancy, obesity, external strabismus, high nasal bridge, high and narrow palate, prominent incisors, tapering fingers and talipes equinus. The probability of the twins being identical was calculated to be 99.999%, using blood types and DNA polymorphism. The following mechanism was considered to explain the discordance in phenotype between the identical twins: first the twins inherited a defective allele responsible for Cohen syndrome from one carrier parent, and next at the early stage of mitotic cell divisions in the patient a mutation occurred in the other allele which resulted in homozygosity for the defective gene for Cohen syndrome.

C-47

POLYMORPHISMS AND LOCATION OF THE HUMAN SYNDECAN -2 GENE FOR COHEN SYNDROME. Yuji MORIMOTO, Fumie TAKAKUBO, Kazuhiro SHIGEMOTO, Ikuko KONDO(Dept. of Hygiene, Ehime Univ, Ehime)

Polymorphisms in the human syndecan-2(SND-2) gene will be useful in the linkage analysis to identify responsible gene(s) for connective tissue diseases. We identified two single base polymorphisms, a ⁴³⁵C-T and a ⁴⁶³C-G substitutions by sequencing PCR products of the genomic DNA. These polymorphisms are located at 5' untranslated region of the SND-2 cDNA, and the estimated heterozygosities in Japanese are 0.43 for ⁴³⁵C-T polymorphism and 0.46 for the ⁴⁶³C-G polymorphism. The SND-2 gene was assigned in the genetic distance of approximately 2cM from DNA marker D8S546(lod score =4.214, $\theta=0.02$) centromeric to D8S1762 at the Cohen syndrome(CHS1) locus. Then, we performed linkage analysis of a consanguineous CHS family using these polymorphism. While parents and an unaffected sister were heterozygous for the ⁴³⁵T⁴⁶³G and the ⁴³⁵C⁴⁶³C allele, but two sibs with CHS1 were homozygous for the ⁴³⁵T⁴⁶³G allele. These results suggest that the SND-2 gene can be one of the candidate genes for CHS1.

C-48

ANALYSIS OF CHROMOSOMAL BREAKPOINT IN A PATIENT WITH BLEPHAROPHIMOSIS SYNDROME BEARING t(3;4)(q23;p15.2). Yumi NAKAGAWA^{1,2}, Johji INAZAWA¹, Yoshimitu FUKUSHIMA³, Yuusuke NAKAMURA⁴, Zenshirou ONOUCHI², Tatuo ABE¹(Dept. of Hygiene¹ and Division of Pediatrics, Children's Research Hospital², Kyoto Pref. Univ. of Med., Dept. of Hygiene, Shinshuu Univ.³, Dept. of Lab. of Mol. Med., Inst. Med. Sci., Univ. of Tokyo⁴)

Blepharophimosis syndrome(BPES) is an autosomal dominant disorder, and involves blepharophimosis, ptosis and epicanthus inversus. Some recent reports document that BPES maps to 3q23. We analyzed the patient of BPES with translocation between 3q23 and 4p15.2. We performed two-color FISH on prophase chromosomes to determine the order of cosmid markers localized on 3q23. The order was provided as follows:cen-1281-1152-1178-937-607-ter. We performed two-color FISH of these markers on the patient's chromosomes. CI3-1281 and 1152 are proximal and CI3-1178,937,607 are distal to the breakpoint, indicating that the breakpoint lies on the region between CI3-1152 and 1178. We are proceeding with genetic analysis of BPES by using these two cosmid markers.

C-49

THE BRAIN FINGER PROTEIN GENE (ZNF179), A MEMBER OF THE RING FINGER FAMILY, MAPS WITHIN THE SMITH-MAGENIS SYNDROME REGION IN 17p11.2. Toshiyuki KIMURA¹, Yoshiki ARAKAWA¹, Satoshi INOUE², Yoshimitsu FUKUSHIMA³, Ikuko KONDO⁴, Kumiko KOYAMA⁵, Masami MURAMATSU², Yusuke NAKAMURA⁵, Tatsuo ABE¹, and Johji INAZAWA¹. (¹ Dept. Hygiene, Kyoto Pref. Univ., Kyoto. ² Dept. Biochem., Saitama Med. school, Saitama. ³ Dept. Hygiene, Shinshu Univ., Nagano. ⁴ Dept. Hygiene, Ehime Univ., Ehime. ⁵ Labo. Mol. Med., Ins Med Science, Tokyo Univ., Tokyo.)

Smith-Magenis syndrome (SMS) is a congenital chromosome-abnormality associated with visible microdeletions of 17p11.2. The features of SMS include the complex phenotypic aberrations such as developmental and growth delay, facial abnormalities, unusual behavior and sleep problems. The complex phenotype may be due to a haploinsufficiency of several contiguous genes. The human brain finger protein gene (ZNF179), a member of the RING finger protein family, has been isolated and mapped to chromosome 17p11.2. FISH analyses of metaphase or interphase chromosomes of six patients with SMS, revealed that ZNF179 gene was deleted in one of both homologous chromosomes 17p11.2, indicating possible association of the defect of this gene with the pathogenesis of SMS. Furthermore, using a prophase FISH ordering system, we sublocalized ZNF179 proximally to LLGL1 which lies on the critical region for SMS.

C-50

CA REPEAT ANALYSIS OF DUCHENNE MUSCULAR DYSTROPHY BY THE FLUORESCENCE PCR.

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[Purpose] Dinucleotide repeat polymorphism is useful for linkage analysis because it detects high polymorphisms compared to PCR-RFLP analysis. We examined the efficacy of 8 microsatellite markers within the DMD gene. [Materials and Methods] Genomic DNA was extracted from blood cells of the 44 normal Japanese females by the standard method. 8 polymorphic regions were amplified by the PCR using primers labeled with fluorescence (three colors). PCR products were electrophoresed on 6 % polyacrylamide sequencing gels using 373S auto sequencer (Parkin Elmer) and analyzed by Genescan Software 672. [Result] Number of alleles and observed heterozygosities of 8 regions were: DYS I; 3/29.5% (no. of X chromosomes=88), DYS II; 10/54.5% (n=88), DYS III; 5/57.6% (n=80), STR44; 10/69.9% (n=88), STR45; 15/76.7% (n=86), STR49; 19/67.4% (n=86), STR50; 11/59.1% (n=88), 3'CA; 4/34.9% (n=86). High total P.I.C. was obtained. [Conclusion] CA repeat analysis by the fluorescence PCR is effective in prenatal analysis and carrier diagnosis of DMD because it is safe and convenient in handling and gives high diagnostic applicability.

C-51

ADHALIN GENE MUTATIONS AND THE CLINICAL HETEROGENEITY IN PATIENTS WITH PRIMARY ADHALINOPATHY. Takeori ENDO, Hisaomi KAWAI, Makoto KUNISHIGE, Hideki MINE, Masashi AKAIKE, Takao MITSUI, Yoshihiko NISHIDA, Shiro SAITO (Dept. 1st. Int. Med., Sch. Med., Univ. Tokushima, Tokushima), Katsuhito ADACHI (Dept. of Neurology, National Sanatorium Tokushima Hospital, Tokushima), Yoshihiro YONEDA, Masahito MIYAZAKI, Toshinori HASHIMOTO (Dept. Pediatr., Sch. Med., Univ. Tokushima, Tokushima), Itsuro HIGUCHI, Hidetoshi FUKUNAGA and Mitsuhiro OSAME (Dept. 3rd. Int. Med., Kagoshima University, Kagoshima)

We have studied the clinical heterogeneity among patients with primary adhalinopathy. Twelve personally examined patients from 6 families and 21 patients from 15 families reported by other groups are included. Mutation in the adhalin gene (point mutation, deletion or insertion) was confirmed in each family. The mean age of onset was 7.5 ± 4.9 yr (range, 1-23 yr) and loss of ambulation was 13 ± 4.3 yr (range, 8-21 yr). There was significant heterogeneity of clinical features even among patients carrying the same mutation, indicating the possible modification by environmental factors.

C-52

TRINUCLEOTIDE REPEAT TRANSCRIPTS IN NUCLEI OF LYMPHOID CELL LINES (LCLs) FROM MYOTONIC DYSTROPHY PATIENTS. Fumiko SAITO-OHARA¹, Toru KOJO², Kanako GOTO², Kiichi ARAHATA², and Tatsuro IKEUCHI¹ (¹Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo, ²Dept. Neuromusc. Res., Natl. Inst. Neurosci., NCNP, Tokyo)

In myotonic dystrophy (DM), a trinucleotide (CTG) expansion in the myotonin protein kinase gene (MTPK) results in the pathological phenotype. In the present study, we analyzed the localization of MTPK gene transcripts in nuclei of LCLs established from 8 DM patients and 8 normal individuals by ISH (*in situ* hybridization) method. The oligonucleotide probes of the sense (CTG-30) and antisense (CAG-30) repeat were labeled with FITC and texas red (TR) respectively, and the nuclei-spreading slides were prepared under the RNase free condition (Taneja et al., 1995). The results showed that TR signals of CAG-30 probe distributed remarkably in the nuclei of DM LCLs, and no signals in the normal nuclei. FITC signals, however, were absent in both the DM and normal nuclei. In contrast, when DNA in the interphase nuclei was denatured before the hybridization, the FITC signal was observed at the only one site and was co-localized with a TR signal in DM nuclei. These findings showed that the antisense probe hybridized to both the MTPK DNA and its transcript and that MTPK transcripts present as foci of nuclear aggregation in the DM cells may have functional significance in the pathogenesis of myotonic dystrophy. We suggest that the method presented here is applicable to a rather wide range of cultured cells from the patients, and might be of practical use for diagnosis of the affected state.

C-53

ANALYSIS OF MUSCLE DYSTROPHIN mRNA IN DMD/BMD PATIENTS WITH DNA DELETIONS.

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(Dept. Pediatr., Tokyo Women's Medical College, Tokyo)

For the purpose of analyzing the relationship between clinical phenotype and changes at the molecular genetic level, we examined muscle dystrophin mRNA from Duchenne(DMD) and Becker(BMD) patients with internal deletions in the DMD gene.

Muscle mRNA was extracted by the AGPC method. Dystrophin mRNA was amplified in three different regions (deletion containing area upstream and downstream) by RT-PCR. The deletion containing area was sequenced with an ALF sequencer.

We found the quantity of muscle mRNA in the DMD cases to be significantly lower than that in the BMD cases ($t=8.33>4.073$, $n=15$, $p<0.001$). The deletions in skeletal muscle mRNA were consistent with those in lymphocyte DNA in 4 BMD and 9 DMD patients, findings compatible with the reading frame theory. As RT-PCR products were not amplified in one DMD case, a transcription failure was assumed. The other DMD case, who had a deletion of exon(E) 48-52 in lymphocyte DNA, died of rhabdomyosarcoma. We detected a deletion of 15bps in the 3' region of E48, alternatively spliced to E53.

Thus, we identified one DMD case in whom the deletion of muscle dystrophin mRNA was not consistent with that of lymphocyte DNA.

C-54

GENE LOCUS FOR AUTOSOMAL RECESSIVE DISTAL MYOPATHY WITH RIMMED VACUOLES MAPS TO CHROMOSOME 9. Takeshi IKEUCHI, Masaaki SAITO, Hajime TANAKA, Sari HIGUCHI, Keiko TANAKA, Shoji TSUJI (Dept. Neurol., Brain Res. Inst., Niigata Univ., Niigata), Tomoya ASAKA, Masaharu TAKAMORI (Dept. Neurol., Kanazawa Univ., Kanazawa), Kyoko SAIDA (Dept. Neurol., Utano Hospital), Eiichiro UYAMA (Dept. Neurol., Kumamoto Univ., Kumamoto), Hidenobu MIZUSAWA (Dept. Neurol., Tsukuba Univ., Tsukuba), Nobuyoshi FUKUHARA (Dept. Neurol., Saigata Hospital, Niigata), Ikuya NONAKA (Dept. Ultrastructural Res., National Center Hospital for Nervous, Mental and Muscular Disorders, Tokyo)

Distal myopathy with rimmed vacuoles (DMRV) is an autosomal recessive neuromuscular disorder, characterized by weakness of distal muscles in lower limbs beginning in early adulthood. Characteristic features of the histopathological findings include numerous rimmed vacuoles, amyloid deposits, eosinophilic inclusions, and atrophic muscle fibers with the notable absence of inflammation. Hereditary inclusion body myopathy (HIBM) with autosomal recessive trait, which was described in Persian Jews, presents clinical and histopathological features resembling those of DMRV. The gene for HIBM has recently been mapped to chromosome 9p-q1.

To test whether DMRV and HIBM map to the same chromosomal region, we performed linkage analysis on seven Japanese pedigrees using microsatellite markers including D9S319, D9S43, D9S248, D9S165, D9S50, D9S276, D9S15, D9S273, D9S166, and D9S175 on chromosome 9. Significantly high lod scores were obtained at three markers: D9S248 ($Z_{max} = 5.90$, $\theta = 0$), and D9S43 ($Z_{max} = 5.25$, $\theta = 0$), and D9S248 ($Z_{max} = 4.23$, $\theta = 0$). Haplotype and multipoint linkage analyses as well as observation of obligate recombination events indicate that the DMRV gene is most likely to be located in the interval of 23.3 cM between D9S319 and D9S276. Localization of the DMRV and HIBM genes in the same chromosomal region raises the possibility that DMRV and HIBM are allelic diseases.

C-55

THE PAX 2 MUTATION IN PATIENT WITH OPTIC NERVE COLOBOMA, HYPOPLASTIC KIDNEY AND MILD MENTAL RETARDATION. Kiyoshi IMAIZUMI, Takuma ISHII, Mitsuo MASUNO, Yoshikazu KUROKI (Dept. Med. Genet., Kanagawa Children's Medical Center) and Eihiko TAKAHASHI, Kunihiko AKAGI (Div. Infec. Immunol. and Rheumatol., Kanagawa Children's Medical Center)

PAX2 is one member of a family of paired box (PAX) gene encoding transcriptional regulators involved in fetal development. PAX2 was shown to be mutated in patients with eye and kidney abnormalities. Patient is a Japanese 11-year-old boy born to healthy unrelated parents after uncomplicated pregnancy. He was pointed out poor weight gain and severe proteinuria at age 1 month. At age 2 years, he was referred to us because of renal failure and he had continuous ambulatory peritoneal dialysis. Bilateral optic nerve coloboma, choroid-retinal degeneration were detected. His visual activity was severely disturbed. By ultrasound, he had bilateral hypoplastic kidneys. At age 9 he was diagnosed as epilepsy because EEG showed abnormal spike and wave complex during sleep. He had mild mental retardation but MRI and CT examination showed no remarkable change. Each of 12 exons of PAX2 were amplified by PCR and the products were analysed by single strand conformation polymorphism (SSCP). Abnormal SSCP patterns were observed in exon 2 and a deletion of 22 nucleotides by direct cycle sequencing. PAX 2 mutations were detected in ten patients up to now. Six of the mutations were detected in exon 2 and they suggested a common mutational mechanism.

C-56

LOCALIZATION OF A 10q BREAKPOINT WITHIN THE PAX2 GENE IN A PATIENT WITH A *DE NOVO* t(10;13) TRANSLOCATION AND OPTIC NERVE COLOBOMA-RENAL DISEASE. Koji NARAHARA, Shigeru ITO, Yuji YOKOYAMA (Dept. Pediatr. Okayama Univ. Okayama), Michael R Eccles (Dept. Biochem. Univ. Otago), Elizabeth BAKER and Grant R SUTHERLAND (Dept. Cytogenet. Mol. Genet. Women's and Children's Hospital, Adelaide)

We describe a 5-year-old boy with a *de novo* t(10;13) translocation and optic nerve coloboma-renal disease (ONCR). On the basis of GTG-banding analysis of prometaphase chromosomes, the patient's karyotype was interpreted either 46, XY,t(10;13)(q24.3;q12.3) or t(10;13)(q25.2;q14.1). FISH studies using a YAC clone containing the PAX2 gene and YAC clones adjoining FRA10B at 10q25.2 showed that the 10q breakpoint had occurred just within the PAX2 gene and was proximal to FRA10B. These FISH results suggest that the translocation causes a disruption of the PAX2 gene and leads to ONCR, in agreement with the recent reports of PAX2 mutations in two unrelated families with ONCR. We refined the regional mapping of the human PAX2 gene to the junction of bands 10q24.3 and 10q25.1.

C-57

High Resolution Mapping of a Region Spanning a Translocation Breakpoint at 14q13 in a Patient with Tetramelic Mirror-Image Polydactyly. Naomichi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Hirohumi OHASHI (Saitama Child. Med. Ctr., Iwatsuki), Yoshimitsu FUKUSHIMA (Dept. Hyg., Shinshu Univ. Sch. Med., Matsumoto)

Mirror-image polydactyly is a rare type polydactyly in which the ulnar side is symmetrically duplicated without the thumb. The cause of the disorder is unknown although many genes involving pattern formations of limbs bud have been identified. We reported a male patient with tetramelic mirror-image polydactyly and a karyotype of 46,XY,t(2;14)(p23.3;q13)de novo. To isolate the putative gene(s) for the disease, we searched for DNA clones containing the translocation breakpoint, 14q13. After FISH analysis of 29 CEPH YAC clones at 2p23.3/14q13, a YAC clone, 928b7 spanning the breakpoint at 14q13 was identified and at least 14 YAC clones showed chimerism (44.8%). In this YAC, containing a 1.6-Mb human DNA insert, there are 4 STSs: D14S75, AFM200ZH4, D14S306 and D14S728. BAC clones corresponding to these STSs were isolated by PCR screening, and FISH using these BACs revealed the breakpoint exists between AFM200ZH4 and D14S306. We are now constructing BAC contigs between the two STSs and isolated a BAC clone spanning the translocation breakpoint.

C-58

A 4-Mb CRYPTIC DELETION ASSOCIATED WITH inv(8)(q13.1q24.11) IN A TRICHO-RHINO-PHALANGEAL SYNDROME TYPE I PATIENT WITHOUT MENTAL RETARDATION. Tohru SASAKI, Hidenobu SOEJIMA, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki) and Hidefumi TONOKI (Dept. Pediatr., Lab. Cell Biol., Cancer Inst., Hokkaido Univ. Sch. Med., Sapporo)

We report on an 11-year-old girl with typical TRPS I features without mental retardation and a de novo paracentric inversion of the long arm of chromosome 8 involving bands q13.1 and q24.11. Molecular analysis using FISH and microsatellite/RFLP markers detected a cytogenetically unidentified cryptic microdeletion that spans about a 4-Mb extent between 2 STS markers (AFMB331YA9 and D8S1200) at a region around the distal inversion breakpoint. Although the deletion extent of the patient is large, mental retardation was not present at all. This is a first case of TRPS I with deletion of which both ends were analyzed at the molecular level. The data obtained are useful for defining the location of the putative gene(s) responsible for mental retardation in TRPS II (Langer-Giedion syndrome) or TRPS I patients.

C-59

FAMILIAL 15p TETRASOMY OF EXTRA SUPERNUMERARY MARKER 15 CHROMOSOME ASSOCIATED WITH ROBERTSONIAN TRANSLOCATION BETWEEN CHROMOSOMES 13 AND 14. Nobuhiko HOSHI, Kazuhiko OKUYAMA, Hideto YAMADA, Tadashi SAGAWA, Satoru MAKINODA and Seiichiro FUJIMOTO (Dept. Obstet. Gynecol., Hokkaido University School of Medicine, Sapporo)

We report on cytogenetic and molecular investigation of a novel familial case of 15p tetrasomy of extra supernumerary marker 15 chromosome associated with der(13;14)(q10;q10).

The proband is a 36-year-old married male with severe oligozoospermia and normal intelligence. He and his wife attended to our department to get a genetic amniocentesis because of ICSI pregnancy and advanced maternal age (39-year-old). The chromosome analysis of cultured amniocyte colonies showed a Robertsonian translocation between chromosomes 13 and 14: 45,XX,der(13;14)(q10;q10). The abnormal cytogenetic result prompted of further cytogenetic examination in the couple to know the origin of the fetus' translocation. The examination revealed that the fetal translocated chromosome is derived from paternal, and extra small bi-satellited metacentric marker chromosome was observed as a new finding in the father (proband). Although FISH with DYZ3, D13/21Z1, D14/22Z1 probes showed no signal in the marker, two strong D15Z1 hybridization signals were seen on either side of the centromeric heterochromatin. Karyotyping of the proband's parents showed that his mother, who has normal intelligence, carried the same translocation and extra supernumerary marker 15 chromosome, that means the cytogenetic abnormalities are familial inherited 15p tetrasomy associated with Robertsonian translocation between chromosome 13 and 14.

In the light of the results with the conventional cytogenetics, in situ hybridization, and case reports published in literature, the marker chromosome was identified as inv dup (15) without loci such as the Prader-Willi/Angelman syndromes critical regions.

C-60

ANALYSIS OF SRY GENE IN CASE OF AN OVARIAN SERTOLI-LEYDIG CELL TUMOR. Yasuhiko EBINA, Nobuhiko HOSHI, Ritu YAMAMOTO, Takahumi FUJINO, Hitoshi HAREYAMA, Satoru MAKINODA, Seiichirou FUJIMOTO (Dept. Obstet. Gynecol., Hokkaido University, Sapporo)

Sertoli-Leydig cell tumors (SLCT) of the ovary are composed primarily of testicular-type cells, and are associated with virilizing manifestation in approximately 30-40% of cases.

A 47 year old woman with a history of abdominal distension was admitted and underwent a laparotomy. Histopathological examination showed a moderately differentiated SLCT. She had no evidence of defeminization or masculinization and her karyotype was 46, XX. Immunostaining for testosterone was negative. SRY gene was not amplified by PCR analysis of the tumor. Further consideration will be needed to know the histogenesis of SLCT.

C-61

SYNERGISTIC EFFECTS OF ANGIOTENSINOGEN GENE AND ANGIOTENSIN II TYPE 1 RECEPTOR GENE FOR PREGNANCY-INDUCED HYPERTENSION. Gen KOBASHI*, Akira HATA*, Koichi SHIDO*, Seiichiro FUJIMOTO**, and Kiyotaro KONDO* (*Dept. of Public Health, **Dept. of Obstet. and Gynecol., Hokkaido Univ. School of Med., Sapporo, Japan)

To clarify synergism of genetic factors for pregnancy-induced hypertension (PIH), 87 cases and 167 controls were studied. A variant of angiotensinogen (AGT) gene (Met²³⁵ → Thr) in the exon 2, and that of angiotensin type 1 receptor (A1R) gene (A¹¹⁶⁶ → C) in 3'-untranslated region were analyzed by PCR and hybridization. Between PIH and controls, Significant differences were found in frequencies of TT genotype of AGT gene (79% vs. 57%, p<0.001), and in those of AC+CC genotype of A1R gene (18% vs. 8%, p<0.05). After a stepwise regression analysis, both factors were screened, disclosing that AGT gene and A1R gene are independent risk factors in PIH. Compared with a reference group with none of the risks, those with those 2 risk factors were 8.2 times more liable to PIH in a multiple logistic model. It may give a clue to elucidate the gene-environment interaction, and to establish an effective individualized prediction and prevention of PIH, by eliminating manageable risk factors in lifestyles, particularly among the inherited high-risk individuals.

C-62

REGULATION OF THE ERYTHROPOIETIN GENE BY GATA TRANSCRIPTION FACTORS.

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A molecular basis for hypoxia signaling and erythropoietin (Epo) gene regulation was studied. The GATA consensus motif was identified in the -30 region of the Epo promoter. Systematic electrophoretic mobility shift assays using competitors and monoclonal antibodies indicated that GATA transcription factors of GATA-2 and GATA-3 bound the motif. Transactivation assays showed that GATA-2 and GATA-3 could not affect Epo gene expression in normoxia. In contrast GATA-2 repressed and GATA-3 activated Epo gene expression in hypoxia, indicating that GATA-3 functioned as a hypoxia-activated transcription factor.

C-63

HUMAN PYRUVATE KINASE EXPRESSION IN TRANSGENIC MICE: ANALYSIS OF REGULATORY GENE ELEMENTS RESPONSIBLE FOR ERYTHROID-SPECIFIC EXPRESSION.

Hitoshi KANNO, Shiro MIWA (Okinaka Memorial Institute for Medical Research, Tokyo), Hisaichi FUJII (Tokyo Women's Medical College, Tokyo), Tamio NOGUCHI, Tsutomu KOIZUMI (Fukui Medical School, Fukui), Masaru OKABE, Yasuhide YOSHIMURA (Research Institute for Microbial Diseases, Osaka University, Osaka)

Pyruvate kinase (PK) has two structural genes in mammals. We previously showed that a 300-bp R(red blood cell)-type PK promoter was sufficient for transcriptional activity in both K562 cells and murine erythroleukemic (MEL) cells. To investigate regulatory gene sequence(s) responsible for erythroid-specific expression of human R-PK, we prepared a human PK minigene construct and established four lines of transgenic mice. The construct (7.5kb) contained following gene sequences; entire coding sequence of the human R/L(liver-type)-PK, the R- and L-PK promoter, intron 1, 2 and 11, the 3'-flanking region, and the upstream DNase-I hypersensitive (HSS) region, which have been identified in K562 cells. By RT-PCR assay, human R-PK transcripts were unable to be detected in bone marrow of transgenic mice except a single transgenic line. The R-PK mRNA was also detected in liver but not in muscle of the line, suggesting that the minigene might lack elements responsible for erythroid specificity.

C-65

POSSIBLE ASSOCIATION BETWEEN PRESENILIN1 LOCUS AND LATE ONSET SPORADIC ALZHEIMER DISEASE. Yumiko NISHIWAKI, Kouzin KAMINO, Aoi YOSHIIWA, Tetsuro MIKI, Toshio OGIHARA (Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka) and Hideki YAMAMOTO, Toshiko KOBAYASHI (Osaka Municipal Kohsaiin Hospital, Osaka)

Fifty per cents of the risk for late onset sporadic Alzheimer disease (SPAD) has been attributed to $\epsilon 4$ alleles at the APOE gene. To evaluate other risk factors of SPAD, we compared allele frequencies of three loci located at 14q24.3; a polymorphism at intron 2 of FOS gene, D14S43 and a polymorphism at intron 9 of presenilin 1 (PS1) gene between SPAD group and normal control group. D14S43, FOS and PS1 loci showed no association with early and late onset SPAD. However, among non-APOE- $\epsilon 4$ bearers, a significant association was found between the polymorphism of PS1 gene and late onset SPAD ($p=0.016$). These results indicated that the PS1 gene could be related with pathogenesis of some form of late onset SPAD.

C-66

APOLIPOPROTEIN E GENOTYPE IN THE HEALTHY POPULATION: FREQUENCY OF GENOTYPE AND A RISK EVALUATION FOR ALZHEIMER DISEASE BY META-ANALYSIS. Masao KANAMORI, Minoru SUGITA, Takashi IZUNO (Dept. Environ. Health, Toho Univ. School of Med., Tokyo), Kimiko KOBAYASHI, Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med., Univ. Tsukuba, Tsukuba) and Kiyotaro KONDO (Dept. Public Health, Hokkaido Univ. School of Med., Sapporo)

The frequencies of Apolipoprotein E(apoE) genotype was surveyed from samples representative to the population. The risk for Alzheimer's disease(AD) was evaluated by means of meta-analysis, a set of statistical tools for combining and integrating the results of independent studies of the community-based prevalence surveys of AD in Japan and in Europe. 235 people were selected from age 50 to 75 randomly in Iwate prefecture. The probabilities of developing AD according to genotype were estimated. The frequencies of E4 allele were 0.083 for aged 50-64 and 0.104 for aged 65-74. Our results on the allele frequencies were consistent with our Japanese hospital survey. Lower frequency of E4 allele was cleared in Japanese elderly population compared to Caucasian population. The probabilities of developing AD with or without apoE E4 allele were increased with age, and lower in Japanese than in Caucasians. These results confirm the influence of genetic factors and some environmental factors on the prevalence rate of Alzheimer's disease.

C-67

CHROMOSOMAL MAPPING OF PRESENILIN 1 AND PRESENILIN 2 GENE BY FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH).

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The causal genes for early-onset familial Alzheimer's disease(AD) were identified as presenilin 1(PS-1) on chromosome 14 and amyloid precursor protein (APP) on chromosome 21. Presenilin 2 (PS-2) was identified as the third causal gene on chromosome 1. We present its precise physical mapping of PS-1 and PS-2 by fluorescence *in situ* hybridization (FISH). The genomic DNA (18kb, 17kb insert) encoding PS-1, PS-2 were biotinylated for probes. To suppress the repetitive DNA sequences, 10⁵-50 times excess amount of human Cot-1 DNA were added to the probe DNA. The PS-1 gene and PS-2 gene were localized to 14q24.3 and 1q42.1, respectively.

C-68

AUTOSOMAL DOMINANT EARLY ONSET DEMENTIA IN A JAPANESE FAMILY: CLINICAL AND GENETIC STUDIES

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We report the results of clinical and genetic studies of autosomal dominant dementia in a Japanese family. Twenty-two individuals in this family were examined clinically (17 living, 5 deceased) and genetically (16 of 17 living members). Ten (5 deceased) of 22 individuals had early onset dementia (age of onset: 45.2 ± 12.1 year-old on average) and 4 of them had multiple white matter lesions and brain atrophy on brain MRI without history of brain ischemic attack. Other 4 individuals had abnormal white matter lesions on brain MRI without dementia. Linkage studies for chromosome 1q31-42, 14q24.3 and 21q21 responsible for Alzheimer's disease, chromosome 19p13.1-13.2 for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and chromosome 3 for familial non-specific dementia suggested no specific haplotypes cosegregated with the disease. Apo E genotypes were E2/2 and E2/3 in this family. Clinical, neuroimaging and genetic studies revealed that the disease in this family was distinguished from known familial dementia. This is the first report of a large Japanese family with autosomal dominant early onset dementia and leukoencephalopathy.

C-69

ANALYSIS OF MICROSATELLITE POLYMORPHISM IN THE HLA DQ REGION ABOUT NARCOLEPSY . Tetsuya Satoh,1) , Jun Nakura, 1), Lin Ye,1), Tetsuro Miki, 1), Yutaka Honda, 2), and Toshio Ogihara 1) ((1): Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka: 2): Seiwa Hosp.,Neuropsychiatric Research Institute, Tokyo)

Narcolepsy is a kind of sleep disorders, and has four characteristic symptoms; excessive daytime sleepiness, cataplexy, hypnagogic hallucination, and sleep paralysis. Onset of narcolepsy involves in multifactor. Significant association between narcolepsy patients and HLA-DR2 has been already known by analysis of Japanese population. According to past reports, narcoleptics have DQA1*0102-DQB1*0602 haplotype without exception. In this study, we analyzed two microsatellites (DQCAR,GC) in 103 narcoleptics and 98 controls to narrow down narcolepsy susceptibility region; DQCAR locates between the DQA1 gene and the DQB1 gene. GC locates on the 9th intron of the Vitamin D binding protein gene. We used genomic DNA from peripheral blood for PCR using fluorescent-labeled primers. These PCR products used for fluorescent image detection after electrophoresis. We analyzed the results by chi-squared test. For DQCAR, frequency of a specific allele (allele B) of nine alleles detected was significantly higher in narcoleptics compared to controls; All narcoleptics have one or two of allele B. However, allele frequency of GC showed no significant difference between the two populations.

C-70

ANTICIPATION IN SCHIZOPHRENIA. Jun KOH, Hiroshi YONEDA, Yasuhiro INAYAMA, Yoshihiro KONO, Kohichi HAYASHI, Jun SAKAI, Yasushi INADA, Akio HIMEI, Akihide HAYASHI and Toshiaki SAKAI
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Anticipation describes the increase in disease severity or earlier onset in succeeding generations within families. This phenomenon has been reported to be related with expansion of trinucleotide repeat DNA sequences in some genetic illnesses. We examined age of onset among two generations in 19 unilineal schizophrenic families. The age of onset in younger generation was 20.4 ± 5.1 years old, in elder generation was 36.0 ± 9.4 . The difference was significant between two generations by using Wilcoxon t test ($t=3.41$, $df=18$, $p < 0.005$). We conclude that genetic anticipation occurs in these unilineal schizophrenic families. These finding may implicate the expansion of trinucleotide repeat as a genetic etiology of schizophrenia.

C-71

ANALYSIS OF THE 5'-FLANKING REGION OF DOPAMINE D2 RECEPTOR GENE Ming GAO, Tadao ARINAMI, Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med. Sci., Univ. Tsukuba) and Michio TORU (Dept. Neuropsychiatry, Tokyo Medical and Dental Univ., Sch.Med.)

Motivated by the dopamine hypothesis for schizophrenia, we have searched for functional polymorphisms in the 5'-region of the dopamine D2 receptor gene. Two polymorphisms, A-241G and -141~~Ins/Del~~, were detected. Reporter constructs containing the -141C~~Del~~ allele cloned into a luciferase reporter plasmid drove 32% and 48% expression compared to those containing the -141C~~Ins~~ allele in Y-79 cells and in 293 cells, respectively. The number of spiperone binding site in the postmortem putamen tended to be decreased in individuals who carried the -141C~~Del~~ allele vs. those who did not. In a case-control study, the -141C~~Ins~~ allele frequency was significantly higher in the schizophrenics than in the controls. No significant differences in a luciferase activity expression, in D2 receptor numbers in the postmortem brains, and in the allele frequency between case-control subjects were observed among A-241G genotypes. These results suggest that -141C~~Ins/Del~~ is a functional polymorphism that affects the expression of dopamine D2 receptor in human.

C-72

DIFFERENTIAL GENOME ANALYSIS BETWEEN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA USING THE IGCRC PROCEDURE Takahiro TSUJITA, Norio NIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med.), Yuji OKAZAKI (Dept. Neuropsychiat., Nagasaki Univ. Sch. Med.), Shin-ichi INOUE (BAN-YU Tsukuba Research Institute), and Michio OHISHI (Institute of Industrial Technology, the Ministry of International Trade and Industry)

The recently developed in-gel competitive reassociation (IGCR) procedure is a method for an efficient cloning of altered anonymous restriction fragments. By means of this method, two pairs and one pair of monozygotic twins (MZT) discordant and concordant for schizophrenia, respectively, and one normal MZT pair were analyzed, whether they have different genomic DNA that may be related to the putative schizophrenia locus. After digestion of genomic DNA by *MseI*, we executed the IGCRC procedure three times, and constructed differential genomic DNA libraries. Clones in the libraries were used as probes for Southern hybridization. As a result, although we analyzed about 40% of whole genome to date, no difference has been detected in any of the four twin-pairs analyzed. Now, we intend to study these twins in more detail using other restriction enzymes.

C-73

INFLUENCE OF APOLIPOPROTEIN E GENOTYPES ON PLASMA LIPID AND LIPOPROTEIN CONCENTRATION IN THE HEALTHY POPULATION. Masao KANAMORI, Minoru SUGITA, Takashi IZUNO (Dept. Environ. Health, Toho Univ. School of Med., Tokyo), Kimiko KOBAYASHI, Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med., Univ. Tsukuba, Tsukuba) and Kiyotaro KONDO (Dept. Public Health, Hokkaido Univ. School of Med., Sapporo)

Jarvik GP.(1995) reported that the Apolipoprotein E(apoE) locus E4 allele was associated with both late-onset AD and total cholesterol (TCHO). The E4 allele is associated with elevations in TCHO levels relative to the E3 allele, while the E2 allele has the opposite effect. The E4 allele is also associated with atherosclerotic disease and myocardial infarction. 235 healthy people were selected from age 50 to 75 randomly in Iwate prefecture. We have studied on an association between apoE, lipoprotein(a)(Lp(a)), TCHO and other plasma lipid. Sex and age-adjusted mean values of plasma lipid lipoproteins by apoE genotypes were as follows: Lp(a) 15.6 mg/dl, TCHO 196.7 mg/dl for E2/E3, Lp(a) 18.5, TCHO 197.8 for E3/E3, Lp(a) 23.8, TCHO 208.4 for E3/E4. Lp(a) were significantly different for apoE genotypes (as determined by a Kruskal-Wallis test).

C-74

HIGH DENSITY LIPOPROTEIN REDUCTION AND LIPOPROTEIN LIPASE GENE IN JAPANESE SCHOOL CHILDREN. Kei YAMANA, Hisako YANAGI, Kimiko KOBAYASHI *, Mari TANAKA, Shigeo TOMURA, Shigeru TSUCHIYA, and Hideo HAMAGUCHI *
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A reduction of high density lipoprotein cholesterol (HDL-C) is recognized as an important risk factor for coronary artery disease. Lipoprotein lipase (LPL) is an important enzyme for hydrolysis of TG-rich lipoproteins, and its activity is positively correlated with the plasma HDL-C levels. To investigate the association between LPL gene and reduced HDL-C levels in children, we studied the LPL gene polymorphisms in 114 school-age children with reduced HDL-C levels (< 40 mg/dl) and in 194 control children with normal HDL-C levels using PCR-restriction fragment length polymorphisms (RFLPs). Subjects with reduced HDL-C divided into two groups: 70 subjects with reduced HDL-C and normal triglyceride (TG) levels (< 100 mg/dl, 90 percentile of Japanese school children) (the low HDL / normal TG group) and 44 subjects with reduced HDL-C and elevated TG levels (\geq 100 mg/dl) (the low HDL / high TG group). Frequency of the *Pvu* II +/+ genotype in the low HDL / high TG group was significantly higher compared with the low HDL / normal TG group, $\chi^2=7.49$, $p<0.01$, and the control group, $\chi^2=7.23$, $p<0.01$, respectively. In addition, we found one heterozygous for LPL^{Arita} (deletion of G at base 916 in exon 5, the common mutation of LPL gene in Japanese), among the low HDL / high TG subjects. These results suggested that genetic variations of the LPL gene may contribute to the reduction of HDL-C associated with elevated TG levels.

C-75

QUANTITATIVE TRAIT LOCUS MAPPING OF HUMAN PLASMA CHOLESTEROL CONCENTRATION. ¹Yoshimi IJIMA, ¹Kimiko KOBAYASHI, ²Hisako YANAGI, ³Yae SHIMAKURA, ¹Tomohiro HAYAKAWA, ¹Tadao ARINAMI, ¹Hideo HAMAGUCHI
(¹Dept.Med.Genet.,²Inst.Basic med.²and Inst.Comm.Med.³and Inst.Clin.Med., Univ.Tsukuba)

To map the loci responsible for multifactorial hypercholesterolemia, family studies were done by using genetic markers. We collected samples from 101 Japanese nuclear families ascertained through a child with hypercholesterolemia (≥ 200 mg/dl). There were 422 individuals and 157 sib-pairs. We tested the linkage between plasma cholesterol concentrations and the candidate genes for LDL-receptor, apoB, apoE/C-I/C-II, apoA-I/C-III/A-IV, apo(a), VLDL-receptor, and LPL, by quantitative trait locus methods (QTL methods). We also tested the linkage between hypercholesterolemia and the candidate genes by affected sib-pair methods. For statistical genetic analyses, the program SIBPAL(S.A.G.E.) and SPLINK were used. The linkage between plasma cholesterol concentrations and the apoA-I/C-III/A-IV gene cluster region were observed ($p<0.00008$). In addition, we also observed the linkage between hypercholesterolemia and the apoA-I/C-III/A-IV gene cluster region by affected sib-pair methods ($p<0.02$). As to other candidate loci, no linkage was observed by QTL methods. Thus, these data suggest that there is a gene responsible for plasma cholesterol concentrations and hypercholesterolemia at or near the apoA-I/C-III/A-IV gene cluster region.

C-76

HYPOALPHALIPOPROTEINEMIA DUE TO MUTATIONS OF APOLIPOPROTEIN A-I GENE. Kimiko KOBAYASHI, Hisako YANAGI, Nao YAMAMOTO, Tadao ARINAMI, Shigeru TSUCHIYA, and Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med. Sci., and *Inst. Comm. Med., Univ. Tsukuba, Tsukuba)

To investigate the frequency of the hypoalphalipoproteinemia due to the mutant apo A-I gene, we have analyzed the sequence variation of apo A-I gene from 33 pupils with low HDL-cholesterol (≤ 35 mg/dl) or apo A-I (≤ 100 mg/dl) levels, who have been screened from 1254 school children in a school survey. We have detected three kinds of frameshift mutations, a splicing mutation, and a missense mutation from them. These five mutations were not observed in 61 other pupils with low HDL-cholesterol levels (≤ 40 mg/dl). The findings suggest that the hypoalphalipoproteinemia due to the mutant apo A-I gene is a relatively common autosomal dominant inborn error of metabolism that affects about one in 250~300 peoples. In addition, we have examined the relationship between HDL-cholesterol and apo A-I levels and a -76 G/A polymorphism in the promoter of the apoA-I gene, which was reported to be associated with the apo A-I transcription efficiency *in vitro*. The frequencies of A allele was higher in pupils with low levels of apo A-I (≤ 100 mg/dl) than the other pupils (>100 mg/dl)(0.23 vs 0.12, $p=0.018$). There is, however, no association between the polymorphism and HDL-cholesterol levels. These data suggest the -76 G/A polymorphism appears to regulate apo A-I production *in vivo* but its effect on HDL-cholesterol levels is small, if present.

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X chromosome	C-16	8p12-21	A-66
aberration		9q31	B-46
X-arrestin	A-12	11p15.5	B-70
X-inactivation	S-I-3, B-28	11q23 abnormalities	A-59
X-linked inheritance	A-13	12q partial monosomy	C-19
Xp22.3	B-26	17p11.2	C-49
		21q22.3	B-24
		22q11	C-21
		8993	A-29