Plenary Lectures

PL-1

APOPTOSIS-INDUCING GENES AND THEIR MUTATIONS. Shigekazu Nagata. (Osaka Bioscience Institute, Osaka)

Homeostasis in vertebrates is tightly regulated by cell death as well as by cell proliferation. The death of cells during embryogenesis, metamorphosis, endocrine-dependent tissue atrophy, and normal tissue turnover is "programmed cell death", mediated by a process called "apoptosis" (1).

The anti-human Fas antibody has cytolytic activity against human cell line expressing the Fas antigen (Fas) (2). Molecular cloning of human Fas cDNA indicated that Fas is a cell surface protein belonging to the TNF/NGF receptor family, and it mediates apoptosis (3). A domain of about 80 amino acids homologous to the TNF type I receptor in the cytoplasmic region of Fas is responsible for the apoptotic signal transduction (4), and this domain is called "death domain". The Fas ligand is a type II membrane protein and it is a member of TNF family (5,6). Biding of Fas ligand to Fas rapidly induces apoptosis, indicating that the Fas ligand is a death factor, and Fas is its receptor (7). The Fas ligand is expressed in activated T-cells, and works as an effector of cytotoxic T cells (8).

Chromosomal mapping and genetic analysis of mouse Fas and Fas ligand genes indicated that mouse lymphoproliferation mutation (*lpr*) and generalized lymphoproliferative disease (*gld*) are mutations in the Fas and Fas ligand genes, respectively (6,9). In one allele of *lpr*, an early transposable element (ETn) is inserted in intron 2 of Fas gene, which causes premature termination and aberrant splicing of Fas mRNA(10). In other allele of *lpr*, a point mutation causes a replacement of amino acid in the cytoplasmic region of Fas, and abolishes the activity to transduce the apoptotic signal (9). On the other hand, in *gld*, a point mutation in the receptor-binding domain of the Fas ligand inactivates its activity (6). Since mice homozygous at *lpr* or *gld* locus develop lymphadenopathy, and suffer from autoimmune disease (11), it is likely that the Fas system plays an important role in development of T cells.

In addition to thymocytes, the Fas mRNA is expressed in the liver, heart, lung and ovary (12). Administration of agonistic anti-mouse Fas antibody into mice induced apoptosis in the liver, and quickly killed the mice causing a liver damage (13). These findings suggest an role of the Fas system in programmed cell death in the liver, and a possible involvement of Fas in pathological tissue damage in CTL-mediated autoimmune disease such as fulminant hepatitis.

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PL-2

MOLECULAR ANALYSES OF AZOOSPERMIA AND DRPLA. Shigeo NAGAFUCHI (Natl. Children's Med. Res. Ctr., Tokyo)

We analyzed DNA from 50 Japanese men with azoospermia whose Y chromosome were cytogenetically normal. The presence or absence of every 26 loci on the Y chromosome was examined in each patient. Of these patients 6 had a small interstitial deletions located within the distal part of Yq11. The deletion of the DYS7C locus was found in all 6 patients suggesting that the presence of azoospermic factor (AZF) in the region surrounding this locus.

Dentatorubral and pallidoluysian atrophy (DRPLA) is a progressive neurodegenerative disorder associated with expansion of an unstable CAG repeat. Expansion was usually associated with paternal transmission and repeat size showed a close correlation with age of onset. The gene was ubiquitously expressed to form a single 4.5 kb transcript and encoded by an open reading frame of 1184 amino acids. Although the predicted amino acid sequence does not reveal any function, it does contain interesting motifs consisting of a proline rich sequence and two stretches of arginine-glutamic acid dipeptides.

PL-3

THE JAPANESE SOCIETY OF HUMAN GENETICS AWARD LECTURE MOLECULAR ASPECT OF INHERITED METABOLIC DISORDERS OF AMINOACIDS. — BRIDGING CLINICAL MEDICINE AND MOLECULAR SCIENCE. Ichiro MATSUDA (Dept. of Pediatr. Kumamoto University School of Medicine, Kumamoto)

Recent advance of molecular technology have been facilitated studies on genetic disease. When examing mechanisms involed in such diseases, attention should be directed to amino acids. Phenylketonuria was studied in detail by Woo et al. and our reseach team has done extensive investigation on genetic amino acids related diseases, including urea cycle disorders, maple syrup urine disease, prolidase deficiency and hypertyrosinemia. We clarified the structures of cDNA or genome DNA of the respective genes, and as well as the correspondig mutant alleles. Among them a phenotype-genotype relationship, a standard issue in genetic diseases, was established in case of ornithine transcarbamylase deficiency and maple syrup urine disease. Such studies reveal clearly the mechnisms involved in the normal and pathological events in human physiology.

PL-5b

ALDOSE REDUCTASE AND DIABETIC COMPLICATIONS. Chihiro NISHIMURA (Dept. Pediatric Pharmacol., National Children's Medical Research Center, Tokyo)

Aldose reductase (AR) catalyzes the reduction of various aldehydes including the aldehyde form of glucose to the corresponding sugar alcohol, sorbitol. The involvement of AR in the pathogenesis of various diabetic complications has been implicated for more than two decades. However, it is not yet clear how and to what extent the perturbation of this sorbitol pathway plays a role in the development of cellular damage under diabetic conditions. This led us to generate transgenic mice expressing a high level of human AR in various tissues and study the effect of enhanced sorbitol pathway on the course of specific tissue injuries. To further elucidate the association between tissue AR level and the progress of diabetic complications in a human population, we developed a new enzyme immunoassay system using antibodies raised against recombinant human AR generated in a baculovirus system.

Symposium

S 1

AMYLOIDGENIC PROCESS IN A TRANSGENIC MOUSE MODEL FOR FAMILIAL AMYLOIDOTIC POLYNEUROPATHY. Ken-ichi YAMAMURA (Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder characterized by the extracellular deposition of amyloid fibrils and by prominent peripheral and autonomic nerve involvement. The amyloid protein is mainly composed of variant transthyretin (TTR) with one amino acid substitution, for example, methionine at position 30 (hMet30). The human TTR gene has been cloned and well characterized at molecular level. To elucidate the pathological process of this disease, we have produced transgenic mice by introducing four constructs. Findings from analyses of these transgenic mice are as follows: (1) the amyloid deposition starts at 9 months of age although the serum hMet30 reaches an adult level at 1 month of age, (2) the tetramers composed of mostly mutant TTR are important for deposition due to physico-chemical property of this molecule, (3) serum amyloid P component does not affect the initiation, progression and tissue distribution of amyloid deposition, (4) microenvironment in each tissue, such as rich blood flow and loose tissue structure, can affect the amount of amyloid deposition, (5) the environmental factor, for example living condition, can affect the amyloid deposition.

S 2

DOMINANTLY INHERITABLE CONNECTIVE TISSUE DISORDERS.
Peter M. ROYCE (Terumo Institute of Biomedical Science, Nakai, Kanagawa).

In recent years the molecular basis of several of the major dominantly inheritable disorders of connective tissue, which include osteogenesis imperfecta (OI), most forms of the Ehlers-Danlos syndrome (EDS), several of the chondrodysplasias, and Marfan syndrome, has been elucidated, although this remains to be translated into a proper understanding of their pathophysiology. In almost all individuals with OI the condition results from a mutation in one of the two type I collagen genes, COLIA1 and COLIA2. EDS type IV is caused by mutation in the gene for type III collagen, COL3A1, whilst in EDS VII a failure in processing of procollagen I to collagen occurs as a consequence of COLIA1 or COLIA2 mutations. Mutations in the genes for collagen types II and X have been identified in certain of the chondrodysplasias, and in Marfan syndrome there are mutations in the FBN1 gene for fibrillin, a component of non-collagenous micro-fibrils. Both collagen and fibrillin mutations are expressed in the heterozygous state. In some cases the consequence may be a null allele, whilst in others the main pathogenetic mechanism may be a dominant negative effect exerted by a mutant gene product.

S 3

FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS.
Jun GOTO (Department of Neurology, University of Tokyo, Tokyo)

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of the motor nervous system. Though most cases of ALS are sporadic some families demonstrate clear Mendelian inheritance with high penetrance. ALS trait is inherited with autosomal dominant or recessive manner. A gene causing familial ALS (FALS) has been assigned to chromosome 21q22.1 by linkage analysis. Cytosolic Cu/Zn superoxide dismutase is a homodimer enzyme which catalyses the conversion of superoxide into hydrogen peroxide plus dioxigen. The gene for this enzyme (*SOD1*) maps to the candidate region between *D21S213* and *D21S219*. Mutations in the *SOD1* gene are associated with FALS linked to chromosome 21. Mutations are misssense types in most cases. However, we have recently identified a new frameshift mutation coding a truncated protein in a Japanese family. Molecular and cellular mechanism which leads to motor neuron cell death in FALS carrying *SOD1* mutations remains obscure. Loss-of-function mechanism might allow free radicals to destruct motor neurons. Indeed, SOD1 activities have been reported to be decreased in various tissue of the patients. However, possibility of gain-of-function mechanism cannot be excluded. Transgenic mice carrying human *SOD1* mutations have been reported. They presented a motor neuron disease but the enzyme activities were not decreased.

S 4

RETINOBLASTOMA:MOLECULAR BASIS FOR TUMOR DEVELOPMENT AND FUNCTION OF THE RETINOBLASTOMA GENE. Tomoko HASHIMOTO (Dept. Genet., Hyogo Coll. Med., Nishinomiya) and Rei TAKAHASHI (Dept. Pathol., Kyoto Univ., Kyoto)

Retinoblastoma(RB) is a childhood intraocular tumor inherited in an autosomal dominant fashion. The RB gene, mapped on chromosome 13q14.2, prevents RB development. We analyzesd somatic and tumor cells of two hereditary RB cases for RB-gene mutation by sequencing genomic DNA and cDNA and by detecting the RB protein expression. In each case, an intragenic deletion in one RB allele was found in the somatic cells, whereas the identical deletion was detected in two alleles (two hits) in the tumor cells. Analysis of LOH of RB tumor cells showed that one chromosome 13 with the normal allele was missing and the other chromosome 13 with the abnormal allele was duplicated.

To study RB gene functions, we converted an RB-negative cell line, HTB9, into an RB-positive cell line, H-CL2, by transfection of an RB expression vector. H-CL2 cells showed serum-dependent growth, a prolonged population-doubling time, lack of the ability to form colonies in soft agar, and lack of tumorigenicity in nude mice. At reaching confluency, H-CL2 cells stopped growing and remained attached to culture plates, whereas HTB9 cells detached from the plate. These detached cells were dye-exclusive but showed nuclear fragmentation characteristic of apoptotic cells. These results suggest that RB expression may prevent apoptosis.

S 5

HUNTINGTON'S DISEASE IS A "CAG REPEAT DISEASE" WITH AUTOSOMAL DOMINANT TRAIT. Ichiro KANAZAWA (Dept. Neurol., Inst. Brain Res., Univ. Tokyo, Tokyo)

Huntington's disease is an adult onset neurodegenerative disorder clinically characterized by choreatic involuntary movements and psychiatric problems and pathologically by atrophic features of the striatum with a predominant medium-sized neuronal cell loss. Genetically, the disease is inherited with an autosomal dominant trait. Localization of the gene at the telometric end of the chromosome 4 (4p16.3) was revealed by a linkage analysis using polymorphic DNA markers in 1983. After extensive exploratory studies for 10 years, in 1993 a causative gene for Huntington's disease was found in the expected region and named as IT15. IT15 gene of normal chromosome contains a CAG repeat (6～32) at the 5' end, whereas that of abnormal chromosome has expanded numbers of CAG repeat (40～more than 100). Thus we analysed IT15 gene of normal and Huntington's disease samples in Japanese, and obtained exactly the same results as Western countries. Just following the CAG repeat sequence in the IT15 gene, there is a CCG repeat region; number of which shows polymorphism. According to data obtained from Scotish and Canadian populations, a genotype linked tightly to the disease was 7 CCG repeats (more than 90 %). However, it was 10 repeats (more than 80 %) in Japanese populations. These results suggest that Japanese Huntington's disease gene could be predominantly originated in Japan or imported from somewhere other than the Western countries.

General Contributions

A-1

HUMAN 18TH CHROMOSOME GENE LIBRARY AND GENE MAPPING.

Hitoshi NAKASHIMA,1 Takashi IMAMURA2, Tomoko Hasegawa3, (1.Dept.Intern Med., Kyushu.Univ. Fukuoka, 2.Dept.Hum.Genet., National Institute of Genetics, Mishima.,3.Shizuoka Children's hosp.)

We report herein the results of mapping 60 new cosmid clones on human 18th chromosome by FISH . A cosmid library was constructed from MS126-21 human-rodent hybrid cell line. 600 cosmids containing large human DNA inserts were isolated. Of the 200 cosmids thus far anaysed, a total of 60 cosmids have been mapped to metaphase chromosomes by FISH. To map the probes in terms of conventional cytogenetic bands, cosmid clones were hybridized to either R-banded or DAPI-banded metaphase chromosome spreads. We found that some of those cosmids which we were unable to localize on the R-banded metaphase spreads were clearly mapped on the DAPI-banded chromosome preparations.Four clones of these 60 cosmids, SCW0204G,0306G,0403B and 0407G, which were hybridized on the short arm of 18th chromosome, were used for cDNA cloning. A cosmid DNA was digested by Sau 3AI and ligated to dephosphorylated adaptor. Following to hybridization with human brain cDNA library PCR between adaptor primer and cDNA library vector primer produced genomic/cDNA kimeric fragment. These fragment DNA sequences thought to be useful for cloning full length cDNAs exit on the short arm of 18th chromosome.

A-2

MAPPING OF 5 HUMAN GENES USING FLOW-SORTED CHROMOSOMES AND FISH. Yimin WANG, Shinsei MINOSHIMA, Jun KUDOH, Masayuki AMAGAI, Ryo KUBOTA and Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med.)

Five human genes have been mapped by spot-blot hybridization of flow-sorted human chromosomes and fluorescence *in situ* hybridization (FISH). These genes are listed below in the following format : gene name, (gene symbol), [region] and <collaborators>.

1) Desmocollin 3 (DSC3) [18q12] <K.Green et al.>

2) Desmocollin 4 (DSC4) [18q12] <K. Kawamura et al.>

3) Hepatocyte growth factor activator (HGFA) [4p16] <N. Kitamura et al.>

4) Tyrosinase like protein Pmel 17 (D12S53E) [12q13-q14]

5) Ubiquinone-binding protein (QPC) [8q22] <M. Nishikimi et al.>

A-3

CHROMOSOME MAPPING OF THE NOVEL GENES EXPRESSED IN A HUMAN IMMATURE MYELOID CELL LINE KG-1. Naohiko SEKI, Ken-ichi ISHIKAWA, Takahiro NAGASE, Nobuo NOMURA (Lab. Gene Structure I, Kazusa DNA Res. Inst., Chiba) and Tada-aki HORI (Div. Genet., Natl. Inst. Radiol. Sci., Chiba)

We established a new approach to isolate novel full-length cDNA clones expessed in a human immature myeloid cell line KG-1, predicted their coding sequences and performed computer search of the databases and Northern blot analysis of various human tissues. We have determined the chromosomal assignment of 80 novel genes by studing the segregation of polymerase chain reaction (PCR) products in human-rodent somatic cell hybrids DNA. Furthermore, 50 of eighty assigned genes were determined their precise chromosomal map position using fluorescence *in situ* hybridization (FISH) techniques. These mapped new genes will provide useful expressed sequence markers in the physical mapping of the human genomic region where disease-related linkage have been suggested or are being pursued.

A-4

CHROMOSOMAL MAPPING OF HUMAN CELL CYCLE RELATED GENES BY FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH). Takako TAKANO, Yasuko YAMANOUCHI (Dept. Hygiene & Pub. Health, Teikyo Univ. School of Med.), Akihisa NAGATA and Hiroto OKAYAMA (First Dept. of Biochem., Univ. of Tokyo)

New genes related to the cell cycle control which are expressed mostly in the G2 phase have been isolated from a human fibroblast cDNA library using *S. pombe* mutant as host. These genes named W1-1, Min1 were mapped on the chromosomes by fluorescence *in situ* hybridization (FISH).

Normal human male R-banded prometaphase chromosomes were prepared by thymidine synchronization followed by bromodeoxyuridine incorporation. The cDNAs (3kb, 4.5kb insert) encoding W1-1, Min1 were biotinylated for probes. To amplify the signals from the W1-1, Min1 genes, we used goat antibiotin antibody and fluorescent anti-goat IgG.

The W1-1 gene and Min1 gene were localized to 7p22 and 14q21 respectively.

A–5

CHROMOSOME MAPPING OF NOVEL GENES EXPRESSED IN HUMAN KERATINOCYTES. Yoichi MORISHIMA, Eiichiro UEDA, Kyoko NONOMURA, Keisuke KONISHI, Kiyofumi YAMANISHI, Hirokazu YASUNO (Dept. Dermatol., Kyoto Pref. Univ. of Med., Kyoto) Takeshi ARIYAMA, Johji INAZAWA and Tatsuo ABE(Dept. Hyg., Kyoto Pref. Univ. of Med., Kyoto)

Analyzing 607 clones of cDNAs derived from cultured human keratinocytes by partial sequencing from the 3' end, we got 276 novel cDNAs. Chromosome mapping of these cDNAs was tried by fluorescence in situ hybridization(FISH), to serve as genetic markers in the construction of genetic maps and the isolation of candidate disease genes using genetic linkage analysis. The fluorescent signals of hybridized probes were amplified with avidin-fluorescein isothiocyanate and biotinylated anti-avidin D. After determination of chromosomal localization, these cDNAs were searched by partial sequencing from the 5' end. As a result, we determined chromosomal localization 42 novel cDNAs and 7 cDNAs matched registered human genes.

A–6

PHYSICAL MAPPING OF THE GENES FOR THE 100-kDa COMPLEMENT-ACTIVATING COMPONENTS OF Ra-REACTIVE FACTOR (CRARF AND *Crarf*) TO HSA 3q27-q28 AND MMU 16B2-B3. Fumio TAKADA,[*,†] Naohiko SEKI,[‡] Yoh-ichi MATSUDA,[§] Yoshinaga TAKAYAMA,[*] and Masaya KAWAKAMI[*]
Depts. [*]Mol. Biol. and [†]Pediatr., Kitasato Univ. Sch. Med., Sagamihara; [‡]Lab. Gene Structure I, Kazusa DNA Res. Inst., Kisarazu; and [§]Div. Genet., Nat. Inst. Rad. Sci., Chiba

Human and mouse genes for the complement-activating serine protease component (P100) of Ra-reactive factor, a novel bactericidal factor (CRARF and *Crarf*), were mapped to R-banded metaphase chromosomes by fluorescence *in situ* hybridization with human and mouse P100 cDNA of 2.7 k- and 2.0 k-base long, respectively. The localization of fluorescent signals showed that CRARF and *Crarf* were mapped to human 3q27-q28 and mouse 16B2-B3, respectively. This evidence is consistent with the previous assumption that the distal portion of the long arm of human chromosome 3 is homologous to the proximal portion of mouse chromosome 16.

A-7

FINE MAPPING OF THE PUTATIVE GENE FOR TRANSIENT ABNORMAL MYELOPOIESIS (TAM)

Tohru OHTA, Yoriko WATANABE, Takahiro TSUJITA, Koh-ichiro YOSHIURA, Kyohko ABE, Yoshihiro Jinno, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Yusuke NAKAMURA (Dept. Biochem., Cancer Institute, Tokyo)

TAM is a leukemoid reaction occurring occasionally in Down syndrome (DS) newborn infants and rarely in karyotypically normal infants. We previously confined the localization of the putative TAM gene to a 1 Mb segment between two loci, G51E07 and G52A04, by FISH on a DS patient with inv(21)(q11.2q22.13). We obtained 5 CEPH-YAC clones, 73F6, 852A4, 796F2, 849B10, and 330H1, which cover the G51E07 and the G52A04 loci. As FISH analysis using Alu-PCR products from the YACs as probes was unsuccessful, three YAC clones (73F6, 796F2 and 330H1) were subcloned into cosmids and these cosmid clones containing human genomic DNA were randomly picked up. FISH analysis with the cosmids showed that the proximal breakpoint 21q11.2 was within the 330H1 YAC. We have constructed a cosmid contig covering the breakpoint. Further analysis revealed that the breakpoint is most likely proximal to the 330H1.

A-8

TWO HUMAN GENES ENCODING CELLULAR PROTEINS, RBQ-1 AND RBQ-3, THAT BIND TO THE RETINOBLASTOMA PROTEIN WERE MAPPED TO 16P11.2-P12 AND 1Q32, RESPECTIVELY.

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The RB gene (*RB*) is a tumor suppressor gene whose mutations are observed in a variety of human malignancies, e.g., retinoblastoma, osteosarcoma, small cell lung cancer and others. Previous studies have demonstrated the binding capacity of the *RB* product (pRB) to several cellular proteins including E2F to prevent cell cycle progression. We recently isolated three new distinct cDNA clones for such human proteins. We report here chromosomal localizations of two of these clones, the RBQ-3 gene (*RBQ3*) and the RBQ-1 gene (*RBQ1*), by both polymerase chain reaction (PCR) analysis on a human-hamster hybrid cell panel and chromosome fluorescence *in situ* hybridization (FISH) using phage clones isolated from a human genomic DNA library as probes. The results showed that the *RBQ1* and *RBQ3* were located to 16p11.2-p12 and 1q32, respectively.

A-9

MICRODELETIONS OF CHROMOSOMAL REGION 22q11 IN 4 PATIENTS WITH IDIOPATHIC HYPOPARATHYROIDISM.

Yoshio MAKITA[1], Mitsuo MASUNO[1], Kiyoshi IMAIZUMI[1], Yoshikazu KUROKI[1], Katsuhiko TACHIBANA[2], Hiroki KURAHASHI[3], Isamu NISHISHO[3]. ([1]Div. Med. Genet. and [2]Div. Endocrinol. and Metab., Kanagawa Children's Medical Center, Yokohama. [3]Div. Clin. Genet. Biomedical Research Center, Osaka Univ. Osaka)

Idiopathic hypoparathyroidism occurs with increased frequency in patients with DiGeorge syndrome(DGS). A broad spectrum of clinical severity is seen in the DGS. Recently, haplo- insufficiency in chromosome 22q11 was identified in DGS, velo-cardio-facial syndrome and conotruncal anomaly face syndrome and cases of isolated congenital heart disease. These clinical features are thought to be a heterogeneity of CATCH22 syndrome resulting from 22q11 deletion(J. Med. Genet. **30**:801-802(1993)).

We examined microdeletion of 22q11 in 4 patients with idiopathic hypoparathyroidism by FISH technique. We used three cosmid probes: DO832(kindly provided Dr. S. Halford), cos-71(manuscript in preparation), cHKAD-26 for detection of 22q11 haploinsufficiency.

These loci we used were deleted in all patients. There was no correlation among the patients between the severity of complications and the size of deletion. These results suggested some cases with idiopathic hypoparathyroidism were a part of CATCH22 syndrome and CATCH22 syndrome was not a contiguous gene syndrome.

A-10

ISOLATION OF YEAST ARTIFICIAL CHROMOSOMES FROM HUMAN CHROMOSOME 11q23 REGION.

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A human chromosome region 11q23-specific DNA library has been constructed by means of microdissection-microcloning method with polymerase chain reaction (PCR) technique (Genomics, 16:169-172, 1993). DNA sequences were determined for 25 microclones that contained approximately 300-500 bp insert and gave a unique (single copy) signal in Southern blot analysis. The sequence tagged site (STS) was designed for each microclones and used for yeast artificial chromosome (YAC) (Jpn. J. Hum. Genet., 39: 249-254, 1994). A total of 33 YAC clones have been isolated by using 8 STSs of microclones and 8 known STS located at 11q22-23 region. The chromosomal locations of these YAC clones were determined by fluorescence in situ hybridization (FISH) method with biotinylated DNA probes prepared by LA (Long and Accurate)-Alu-PCR. Physical mapping of YAC clones at ataxia-telangiectasia locus at 11q22-23 region was done by PCR-assay with STS markers. These chromosomal region-specific STSs and YAC clones will be useful in the construction of physical contig map and also in the positional cloning of human genes localized to the q22-23 region of human chromosome 11.

A-11

The Human Interleukin-10 Receptor Gene Maps To Chromosome 11q23.3

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Interleukin 10 (IL-10) is a pleiotrophic cytokine produced by T helper cells (Th2), B cells, macrophages, thymocytes and keratinocytes. IL-10 inhibits production of cytokines by T helper cells (Th1) and macrophages, and also has costimulation effect on the proliferation and differation of B cells, mast cells and T cells. Furthermore, IL-10 enhances the expression of MHC class II antigen on B cells and Fc γ receptor on macrophages, and also down-regulates the expression of MHC class II antigen on monocytes. Thus, IL-10 is an important and negative immune response regulator. The human interleukin-10 receptor (IL-10R), which can bind only human IL-10, is structurally related to interferon receptors (Liu et al.1994). We determined the precise location of the human IL-10R gene by fluorescence in situ hybridization (FISH) with biotinilated 3.5 kb cDNA as a probe. It is concluded that the IL-10R gene maps to chromosome 11q23.3. Related genes, the human genes encoding for CD3 γ , CD3 δ , and CD3 ε have been also mapped to 11q23. This region contains the breakpoints of 11q23 rearrangements in hematopoietic malignancies.

A-12

CHROMOSOME MAPPING OF THE NEWLY IDENTIFIED BILIVERDIN-IX β REDUCTASE GENE (BLVRB) TO 19q13.13->q13.2 BY FISH. Fumiko SAITO[1] Tokio YAMAGUCHI[2], Akihiko KOMURO[3], Takashi TOBE[3], Tatsuro IKEUCHI[1], Motowo TOMITA[3], and Hiroshi NAKAJIMA[2] ([1]Dept. Cytogenet., [2]Dept. Biochem. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ.,[3]Dept. Physiol. Chem., Sch. Pharmaceut. Sci., Showa Univ., Tokyo)

At the final step in heme metabolism in mammals, biliverdin IX was reduced to bilirubin IX by biliverdin reductase. This enzyme is divisible into two classes in human : (1) biliverdin-IX α reductase, which is a major component of human adult bile and whose gene (BLVRA) is mapped to 7p14->cen region using the human-mouse hybrid system; and (2) biliverdin-IX β reductase, a newly purified enzyme, found to be predominant in fetal bile. In this study, we determined the chromosomal localization of the human gene for this β reductase (BLVRB) by fluorescence in situ hybridization (FISH) using a 0.76 kb cDNA fragment as a probe. As a result, a total of 33 doublet fluorescent signals were observed, of which 24 (73%) were detected exclusively in the chromosome region 19q13.1->q13.3. More precisely, 20 of the 24 doublets (83%) were located in the restricted region 19q13.13->q13.2. The other signals were distributed randomly along the chromosomes, with no other signal-clustering regions. Thus, the human BLVRB gene was mapped to chromosome 19q13.13->q13.2.

A-13

DETAILED ANALYSIS OF A t(5;17)(q13;q25) WITH KLIPPEL-FEIL SYNDROME.
Minoru ISOMURA, Shinsuke NINOMIYA Masatoshi INOUE (Dept. Biochem., Cancer Inst.), Yoshimitsu FUKUSHIMA (Div. Genet. Saitama Children's Med. Ctr., Saitama), and Yusuke NAKAMURA (Dept. Biochem., Cancer Inst., Tokyo)

We have analyzed a chromosomal translocation with Klippel-Feil syndrome. Analyses of the patient's chromosomes with a linearly-ordered seven cosmid markers on 17q24-25.1 indicated that the breakpoint is located within two loci defined by cosmids cCI17-509 and cCI17-546. Subsequently, a YAC clone containing both flanking loci was isolated and a cosmid library was constructed from the yeast DNA. Among the cosmid clones isolated from the cosmid library, cosmid 3a detected extra bands in the patient's DNA digested with EcoRI, PstI, or PvuII by Southern hybridization. This result implied that cosmid 3a contains the chromosomal breakpoint on 17q. Using this cosmid DNA, cDNA library screening and exon trapping have been performed to isolate a candidate gene for Klippel-Feil syndrome.

A-14

MOLECULAR GENETIC CHARACTERIZATION OF THE TRANSLOCATION BREAKPOINT ON CHROMOSOME 22q12.2 IN A PATIENT WITH NEUROFIBROMATOSIS TYPE 2 (NF2).
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We previously described a patient with neurofibromatosis type 2 (NF2) who showed a constitutional balanced translocation t(4;22). To characterize the breakpoint on chromosome 22 in this patient in relation to the recently isolated candidate gene (NF2) responsible for NF2, we analyzed DNAs from the patient and her parents using parts of NF2 cDNA as probes.We isolated clones containing DNAs corresponding to the normal chromosome 22 and to the breakpoint in derivative chromosome 4. Southern hybridization analyses and DNA sequencing of the genomic DNAs revealed that the chromosome 22 breakpoint in this patient lies within an intron nearly 800 bp distal to the 3' end of exon 14 of the NF2 gene. The results lend support to the conclusion that NF2 is the gene responsible for the central nervous systemform of neurofibromatosis.

A-15

HIGH-RESOLUTION CYTOGENETIC MAPPING OF THE SHORT ARM OF CHROMOSOME 1 WITH NEWLY ISOLATED 411 COSMID MARKERS BY FLUORESCENCE *IN SITU* HYBRIDIZATION: THE PRECISE ORDER OF 18 MARKERS ON 1p36.1 ON PROPHASE CHROMOSOMES AND "STRETCHED" DNAs.

Takeshi ARIYAMA[1,2], Johji INAZAWA[1], Tatsuo ABE[1], Atsushi HORIUCHI[2], Toshihiko EZAKI[3], Yusuke NAKAMURA[3], Akira HORII[3] ([1]Dept. Hyg., Kyoto Pref. Univ. Med., Kyoto, [2]3rd Dept. Int. Med., Kinki Univ. Med., Osaka, [3]Dept. Biochem., Cancer Inst., Tokyo)

A high-resolution cytogenetic map of the short arm of chromosome 1 with newly isolated 411 cosmid markers was constructed by fluorescence *in situ* hybridization (FISH). These markers were scattered throughout chromosome 1p, but they were preferentially concentrated on R-band dominant regions such as 1p36, 1p34, 1p32, 1p22, and 1p13. Among these markers, 197 were localized on chromosome band 1p36, a region frequently deleted in neuroblastoma. Of these, 18 were precisely ordered on 1p36.1 by multi-color FISH of prophase chromosomes and "Stretched" DNAs as follows; 1pter-163-41-11-1-226-586-568-614-631-665-451-199-190-561-241-74-176-652-1cen. The high-density map of chromosome 1p constructed here can provided useful landmarks for constructing a contig map of the short arm of chromosome 1 with YACs and cosmid clones, and will expedite the identification of breakpoints and/or tumor suppressor gene(s) associated with several types of malignant tumors that frequently exhibit chromosomal aberrations or deletions of chromosome 1p.

A-16

FINE MAPPING OF HUMAN AQP2 AND MIP GENES, TWO MEMBERS OF THE MIP FAMILY, WITHIN CHROMOSOME BAND 12q13 USING TWO-COLOR FISH.

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The human AQP2 (aquaporin 2 gene) encodes a 271 amino-acid protein, and is a member of the MIP (major intrinsic protein of lens fiber) gene family. Recently, we assigned by FISH the locus of AQP2 to 12q13, the site being within the previously mapped region of MIP. In order to further delineate the relative location of this two genes, we performed the refined mapping by the two-color FISH (fluorescence in situ hybridization). Using human genomic DNA clones for AQP2 and MIP as probes, each hybridization signals could be separately identified on interphase nuclei and even on mitotic chromosomes 12 at early stages, but not on more condensed mid-metaphase chromosomes. In 36 (90%) of 40 prometaphase chromosomes 12 observed, hybridization signals of AQP2 were found more proximal than that of MIP within the 12q13 band. These results show that these two genes can be recognized separately, even though both genes belong to the same family. Based on the ability of FISH to resolve loci in early-metaphases, the distance between the locations of AQP2 and MIP was evaluated to be more than 400-500kb. By the single-color FISH performed on the high-resolution banded chromosomes for more refined localization of the two genes, AQP2 localized to 12q13.1 and MIP to 12q13.2->q13.3.

A-17

HIGH-RESOLUTION MAPPING OF 11q23.1-SPECIFIC COSMID MARKERS BY MULTI-COLOR FISH ON PROPHASE CHROMOSOMES AND STRETCHED DNA FIBERS. Tosiyuki KIMURA, Naoki KAKAZU, Johji INAZAWA, Tatsuo ABE (Dept. Hyg., Kyoto Pref. Univ. of Med., Kyoto), and Yusuke Nakamura (Dept. Biochem., Cancer Inst., Tokyo)

The chromosomal region of 11q23 contains cancer breakpoints in several types of malignancies and genes responsible for ataxia-telangiectasia (ATA) and bipolar affective disorder. The construction of a refined physical map in this region is an indispensable step for positional cloning of these loci. We have screened three different YACs from a CEPH YAC library with two cosmid markers, CI11-556 and -420 previously mapped on this region. Of these, a YAC, 913H, was used for the construction of a cosmid library and with these cosmids a cosmid-contig map spanning about 300kb region of 11q23.1 has been constructed by multi-color FISH on both prophase chromosomes and "stretched" DNA fibers. This direct visual mapping system can provide the correct order of adjacent markers as little as 50 kb A refined physical map presented here facilitates the isolation of translocation breakpoints and disease genes mapped on 11q23.

A-18

CONSTRUCTION OF RETINA-SPECIFIC cDNA LIBRARY AND MAPPING OF pmel17 GENE BY FISH METHOD. Ryo KUBOTA[1,2], Yimin WANG[1], Jun KUDOH[1], Shinsei MINOSHIMA[1], Yukihiko MASHIMA[2], Yoshihisa OGUCHI[2] and Nobuyoshi SHIMIZU[1] (Depts. of [1]Mol. Biol. and [2]Ophthalmol., Keio Univ. Sch. of Med., Tokyo)

By combining subtractive hybridization and differential hybridization, we developed a new method to enrich tissue-specific cDNAs. We used human retina cDNA library as a target and human brain cDNA library as a driver. After amplifying inserts with PCR, phenol emulsion reassociation technique was employed to facilitate inserts-hybridization. After hybridization, driver cDNAs were eliminated using streptavidin-attached magnetic beads, and the remaining cDNAs were amplified using PCR and cloned into plasmid vectors. These plasmid clones were harvested in 96-well multi-titer plates and blotted on eight nylon filters containing 1536 clones each. Sublibrary was constructed from the clones which differentially hybridized to the subtracted retina cDNA but not to the brain cDNA. Thirty three clones were isolated as candidates for the retina-specific cDNA and these clones were sequenced, Northern analysis was also performed to determine tissue-expression. To date, we were able to identify four rhodopsin clones, one peripherin clone, one tyrosinase-like protein pmel17 and one as yet unknown cDNA clone which was expressed in both retina and skeletal muscle. Among these pmel 17 gene (D12S53E) was mapped to the band q13-q14 of human chromosome 12.

A-19

MOLECULAR ANALYSIS OF CANDIDATE REGION FOR AN AZOOSPERMIC GENE.

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[1]Department of Human Genetics, School of International Health, University of Tokyo, Tokyo. [2] Department of Urology, Osaka University Medical School, Osaka.

We analyzed DNA of Japanese men with either azoospermia or severe oligospermia whose Y chromosomes were cytogenetically normal. In our previous study, 7 of the 50 patients showed deletions involving DYS7C locus on the long arm. Six of the 7 patients showed a common micro-deletion and the father of one of the patients also showed a deletion in seemingly the same region as his son. The breakpoints in the father were still not distinguishable from those of the infertile son. We proposed that the deletion in the father was smaller than those in the son, and that loss of additional loci around the breakpoints caused azooapermia. If a segment is retained in the father and lost in the son, the segment must contain a gene or a part of it crucial to spermatogenesis. In the present study, YAC clones that may cover the breakpoints of the father and his son were selected. We subcloned the inserts of the YACs into cosmid vectors, and constructed a contig of the cosmids. As a consequence of analysis in detail with the combined use of genomic Southern hybridization and PCR analysis, we determined a cosmid clone spanning the breakpoints. Further analysis of the father and the son is now in progress for determination of their breakpoints. On the other hand, by using cosmids in the contig, direct selection of cDNAs from a human testis cDNA library is also in progress.

A-20

ANALYSIS OF Y-SPECIFIC GROWTH GENE(S)

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The comparison between the adult height and those of patients with various diseases implied the presence of a Y-specific growth gene(s).This time we correlated genotype and phenotype in patients with a partial Yq deletion to localyze the growth gene(s).The results suggest that the Y-specific growth gene(s) is on the Y chromosome in the region defined by DYS11 and DYS246.This region corresponds to one of Y-specific regions,so it must contain Y-specific genes.

In order to analyze structure of this region and to isolate the gene(s),we picked out YAC clones which cover the region from the YAC contig by Page et al. and constructed the restriction map of a YAC clone. Now,we are preparing the cosmid library of the YAC clone and trying to clone the gene(s).

A-21

REFINED MAPPING OF A GENE FOR FUKUYAMA-TYPE CONGENITAL MUSCULAR DYSTROPHY: EVIDENCE FOR STRONG LINKAGE DISEQUILIBRIUM. Tatsushi TODA[1], Shiro IKEGAWA[2], Eri KONDO[3], Kayoko SAITO[3], Yukio FUKUYAMA[3], Mieko YOSHIOKA[4], Toshiyuki KUMAGAI[5], Kaoru SUZUMORI[6], Yutaka NAKAHORI[1], Ichiro KANAZAWA[7], Yusuke NAKAMURA[2], Yasuo NAKAGOME[1] ([1]Dept. Hum. Genet, [7]Dept. Neurol., Univ. Tokyo, [2]Dept. Biochem., Cancer Inst., [3]Dept. Pediatr., Tokyo Women's Med. Coll., Tokyo, [4]Dept. Pediatr., Kobe General Hospital, Kobe, [5]Dept. Pediatr. Neurol., Aichi Welfare Center, Kasugai, [6]Dept. Obstet. Gynecol. Nagoya City Univ. Med. School, Nagoya)

Fukuyama type congenital muscular dystrophy (FCMD) is an autosomal recessive severe muscular dystrophy associated with an anomaly of the brain. Following our initial mapping of the FCMD locus to chromosome 9q31-33, we further defined the locus within a region of approximately 5 cM between loci D9S127 and CA246, by homozygosity mapping in patients born to consanguineous marriages and by recombination analyses in other families. We also found evidence for strong linkage disequilibrium between FCMD and a polymorphic microsatellite marker, mfd220, which showed no recombination and a lod score of 17.49. A "111-bp" allele for the mfd220 locus was observed in 22 (34%) of 64 FCMD chromosomes, but it was present in only one among 120 normal chromosomes. This allelic association with FCMD was highly significant (χ^2=50.7, p<0.0001). We suspect that the FCMD gene could lie within a few hundred kb of the mfd220 locus.

A-22

ISOLATION AND MAPPING OF COSMID CLONES ON THE HUMAN CHROMOSE 13. Akira Kuwano[1], Yuji Morimoto[1], Kumiko Koyama[2], Yusuke Nakamura[2], Ikuko Kondo[1] (1. Dept. Hygene, Ehime Univ. Sch. Med., Ehime. 2 Dept. Biochem., Cancer Inst. Tokyo)

Chromosome 13 has been poorly mapped and been paucity of mapping reagents. Wilson disease gene was cloned this year, and a number of diseases were mapped on chromosome 13, including RB1, Duchenne-like muscular dystrophy, Hirschsprung disease, HHHS, Propionic acidemia A. In order to construct the fine physical map of chromosome 13, we constructed a cosmid library from the chinese hamster/ human hybrids cells GM 10898, which contains chromosome 13 as its sole human component. So far, 14 colonies were mapped to; 13q12;25, 46: 13q14; 40, 59: 13q14.3-21; 68; 13q32; 8, 14, 54: 13q33 62, 135: 13q34; 39, 86: 13cen-p12; 16,67. Mapping effort has been continuing for the rest of clones.

A-23

SEQUENCE-TAGGED-SITES (STSs) ON A HUMAN CHROMOSOMAL REGION, 8q11.1, IDENTIFIED FROM COSMID LIBRARIES CONSTRUCTED FROM A HUMAN-SCID MOUSE HYBRID CELL LINE

Yoriko WATANABE, Tohru OHTA, Takahiro TSUJITA, Yoshihiro JINNO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Kensi 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 and increase of sensitivity to ionizing radiation. We previously mapped the putative human gene (*HYRC*) for hyper-radiation sensitivity that is complementary to murine Scid gene to human 8q11.1 by FISH using Alu-PCR products from human-mouse scid radiation hybrids as probes. We constructed cosmid libraries from this human-scid hybrid cell line that harbors human sequences. A total of 16 cosmid clones were isolated and these cosmids were confirmed to be located to the region 8q11.1 by FISH. We further selected 10 unique cosmid clones by means of Southern blot hybridization and sequenced them to construct sequence-tagged- sites (STSs) with appropriate oligonucleotide primers for PCR. These STSs are useful for positional cloning of the HYRC gene.

A-25

cDNA CLONING BY MAGNETIC BEADS METHOD USING COSMID DNAs FROM DOWN SYNDROME CRITICAL REGION.

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We have previously constructed a cosmid library from flow-sorted human chromosome 21 and identified over 1,000 cosmids covering the Down syndrome critical region (DCR:D21S17-D21S55-ETS2). These cosmid clones were divided into 19 subregions of the DCR.

Here, we attempted to isolate cDNA clones using these cosmid clones as probes for magnetic beads cDNA capture method. Inserts of human brain cDNA library were PCR-amplified and hybridized with biotinylated cosmid clone DNA. cDNAs which were hybridized with cosmid DNA pools were selected with Streptavidin-magnetic beads. After extensive washing, cDNAs were eluted, PCR-amplified and cloned into M13 phage vector or pCR^{TM}. When amyloid precursor protein gene was used as a control probe, cDNA was concentrated 5-10 times by one cycle of selection. This method allowed us to isolate a number of cDNA clones which were subjected to sequence analysis. We found about 30% of clones had *Alu* repetitive sequence or rDNA sequence. Further improvement of this method will provide useful cDNA clones for the pathogenic analysis of Down syndrome.

A-26

ISOLATION OF GENES FROM DOWN SYNDROME CRITICAL REGION OF HUMAN CHROMOSOME 21 BY EXON TRAPPING METHOD.

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We have identified 1,003 region-specific cosmid clones using 10 overlapping CEPH-YAC clones covering the "Down syndrome critical region" (DCR: D21S17-D21S55-ETS2) as hybridization probes for screening high-density replica filters (6 chromosome equivalents) prepared from a cosmid library previously constructed from flow-sorted human chromosome 21. These cosmids were assigned to 19 sub-regions in the DCR region. To isolate genes potentially involved in the pathogenesis of Down syndrome, we are now using these cosmid clones for exon trapping analysis. Pooled cosmid DNAs (approximately 10 cosmids/pool) were subcloned into the pSPL3 vector, and used to transfect COS-7 cells. To date, we have isolated more than 30 putative exons and their DNA sequences were determined. We report the initial progress. (We acknowledge D. Cohen, I. Chumakov, K. Osoegawa, and E. Soeda for their participation in the initial stage of this project.)

A-27

MOLECULAR CLONING OF HUMAN THYROTROPH EMBRYONIC FACTOR (TEF).

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Cell types in the mature anterior pituitary gland are defined by trophic factors that they synthesize and secrete. Thyrotrophs synthesize thyroid stimulating hormone (TSH), a heterodimeric glycoprotein consisting of an α subunit and a β subunit, and regulates thyroid gland growth and hormone production. The β subunit (TSH β) is specific to TSH, while the α subunit is shared by other pituitary hormones. Congenital TSH deficiency is classified into two types; one is isolated TSH deficiency and the other is associated with multiple pituitary deficiencies. Some patients with isolated TSH deficiency had abnormalities of the TSHß gene while others had no such abnormality. The expression of TEF, a basic-leucine repeat transcription factor, was reported to correlate spatially and temporally with the onset of TSHß expression in the developing pituitary gland. TEF was also shown to uniquely activate TSHß expression in transfection analysis (Drolet et al, 1991). As some patients with isolated TSH deficiency might have a genetic abnormality related to TEF, we cloned and sequenced cDNA encoding human TEF. The cDNA was reverse-transcribed from total RNA of human pituitary gland and amplified by PCR with primers corresponding to rat TEF. After subcloning this fragment to a plasmid vector, the sequence was determined and characterized. Human TEF cDNA encodes a polypeptide of 261 amino acids and shows extensive homology with rat TEF. This cDNA may prove useful for studies on patients with genetic abnormalit(ies) in pituitary functions.

A-28

ASSIGNMENT OF THE BREAKPOINTS OF CAT EYE SYNDROME MARKER CHROMOSOMES AND CONSTRUCTION OF REGION (22pter-q11) SPECIFIC COSMID LIBRARY.

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Cat eye syndrome (CES) is caused by tetrasomy of the 22q11. The morphological anomalies in CES include coloboma of the eye, imperforate anus and heart malformation. Most (>90%) of the CES patient has a supernumerary bisatellited marker chromosome (mar) retaining two copies of the 22pter-q11 region. We applied the flow-sorting technique of chromosomes to examine precise breakpoint of mar's for 2 cases and to construct a region-specific cosmid library. The breakpoints (BP) of 2 cases, CH91-157 and EBO-22, were precisely localized as shown in the following map: pter-cen-S24/S9-S57-($BP_{CH91-157}$)-S36-[S75-S66-S259]-(BP_{EBO-22})-S10-qter, where the commonly deleted region of DiGeorge syndrome is indicated with brackets. This result strongly suggested that critical regions responsible for the pathogenesis of cat eye syndrome and DiGeorge syndrome do not overlap. We have constructed a cosmid library (9,200 clones) from the mar chromosome of CH91-157 cells which is approximately 10-times equivalent to the chromosome size. Twelve 22q11 region-specific cosmids were identified by FISH analysis, and STS's were established from those clones. YAC screening using these STS's are now in progress.

A-29

GeneView**^PLUS^* AND ***Locus-in v.2 : CONSTRUCTION OF A COMPREHENSIVE DATABASE SYSTEM FOR HUMAN GENOME MAPPING.

Shinsei MINOSHIMA and Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo)

Mapping data are being compiled into GDB (Genome Data Base) with international collaborative efforts. Search queries to GDB can be performed *via* network using various methods. The GDB has become an inevitable international resource for the archive of human genome analysis. We have developed new softwares, ***Locus-in v.2*** and ***GeneView**^PLUS^*, which are designed for entry of raw mapping data, integration of various genome maps, and efficient use of GDB data. Both systems operate on a workstation with X-window and have smooth graphical user interfaces.

Locus-in v.2 has the following unique functions: (1) to graphically display chromosome ideograms (800 band-level) and zoom-in to a specific region of interest, (2) to generate sub-windows regarding a specific region for entry and display of raw or published data, and ordered or not-ordered data, (3) to create new breakpoints for boundaries of sub-windows on graphic display, (5) to refer published mapping data from GDB through sub-windows, (6) to show detailed information of each map object by clicking it, and (7) to manage the ownership of data from multiple researchers.

***GeneView**^PLUS^* has the following characteristics: (1) to quickly search GDB data with easy operation, (2) to accept both English and Japanese, (3) to show the result with graphical output using chromosome ideograms, (4) to zoom-in to an interested region, and (5) to show additional information by clicking each map object.

Both systems are demonstrated on workstation at the meeting site.

A-30

ISOLATION AND CHARACTERIZATION OF HUMAN *NRAMP* cDNA.

Fumio KISHI (Center for Gene Research, Yamaguchi University) and Susumu FURUKAWA (Dept. of Pediatrics, Yamaguchi University School of Medicine)

The mouse gene locus *Lsh/Ity/Bcg* regulates macrophage activation for antimicrobial activity against intracellular pathogens. A candidate gene, designated natural resistance-associated macrophage protein gene (*Nramp*), recently isolated from a mouse pre-B cell cDNA library encodes an integral membrane protein that has structural homology with known prokaryotic and eukaryotic transport systems. In the present study, the cDNA for human *Nramp* was isolated by screening a human monocyte cDNA library. The cDNA was 2245 bp in length and coded for a protein of 483 amino acid residues with a molecular weight mass of 52.8 kDa. The deduced amino acid sequence was 89% homologous with that of mouse. Southern blot analysis indicated a single gene for *Nramp* counterpart in the human genome. Northern blot analysis revealed a single species of mRNA of approximately 2.5 kb.

A-31

THE CLONING OF THE BREAKPOINT IN THE CASE OF CAMPOMELIC DYSPLASIA(CMPD) WITH DE NOVO t(12;17) TRANSLOCATION.

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CMPD is a congenital disorder, characterized by bowing of the lower extremities, hypoplastic scapulae, tracheomalacia and talipes equinovarus. Sex reversal has been reported in about half of genotypic males. Recently the disease gene has been located on 17q24-q25. We have cloned the breakpoint in the case of CMPD with de novo 46,XY,t(12;17)(q21;q24 or q25). The breakpoint of the chromosome 17 was decided between cosmid 509 and 546 by FISH . The YAC clone covered the two cosmid clones was isolated, and we made cosmid libraries from it. The pulse field gel electrophoresis of the 546 revealed about 900kb extra band in the patient. We performed cosmid walking from 546 and isolated cosmid clone in which the rearrange band was recognized by Southern hybridization. Three exon-like products were obtained by exon trapping. Testis cDNA libraries were screened with their products and two positive clones were obtained. One of them revealed about 4kb band in testis specifically by Northern hybridization.

A-32

CLONING OF THE RABBIT CORNEAL ENDOTHELIAL cDNA.
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(Department. of Ophthalmology, Juntendo University School of Medicine, Tokyo)

The genetic defect causing corneal dystrophy is still unknown except for an abnormality of the gelsolin gene in lattice corneal dystrophy. We isolated corneal endothelial cDNA clones to try the candidate gene approach for the corneal dystrophy. The rabbit corneal endothelial cDNA library (Yamaguchi et al. J. Biol. Chem., 1989) was employed. Plus minus screening was performed using rabbit corneal and iris total RNA as probes. Ten clones which were positive to the corneal probe and negative to the iris probe were obtained. The insert of clones was amplified by PCR and sequenced. One clone showed high homology with the cDNA of B22 subunits of bovine mitochondrial NADH - ubiquinone oxidoreductase and suggests to be the cDNA of B22 subunits of rabbit mitochondrial NADH - ubiquinone oxidoreductase. Another two clones showed no homology with any previous reported DNA and further study is now on going.

A-33

CANDIDATE GENE APPROACH OF RETINITIS PIGMENTOSA.
Keiko FUJIKI, Yoshihiro HOTTA, Mutsuko HAYAKAWA, Marilou G. NICOLAS, Hitoshi KISHISHITA, Misako TAKEDA, Atsushi KANAI (Dept. of Ophthalmol., Juntendo Univ., Tokyo), Masaru YOSHII and Akira MURAKAMI (Natl. Defense Med. College, Tokorozawa)

We have tried to find the mutation of the gene caused retinitis pigmentosa (RP) by candidate gene approach, and found a single base pair substitution (AAT→AGT) leading to a serine - for - asparagine in the rhodopsin gene of a patient with autosomal dominant retinitis pigmentosa (ADRP). Her clinical findings were consistent with sectorial RP as well as a pedigree with the same mutation (Asn 15 Ser) found in Australia (Sullivan LJ et al., 1993). On the other hand, mutation of codon 174 in the rhodopsin gene found in two patients among 36 unrelated autosomal recessive RP (ARRP) did not cosegregate with the disease in spite that the substitution of AGC→GGC led to a serine - for - glycine and was not found in 40 unrelated ADRP patients and 48 normal subjects. We have also detected the polymorphisms in the peripherin/*RDS* gene, among which the mutation of codon 304 has the substitution of amino acid (Glu304Gln).

A-34

THE DIGENIC INHERITANCE PATTERN OF UNLINKED ROM1 AND PERIPHERIN/RDS LOCI IN PATIENTS WITH RETINITIS PIGMENTOSA. Kazuto Kajiwara (Department of Neurobiology, Stanford University)

We reported three mutations in peripherin/*RDS* gene in families with autosomal dominant retinitis pigmentosa (RP) (Kajiwara, *et al*., *Nature*, 1991). One of these mutations, termed Leu185Pro, was found in 2 additional families. As we analyzed these families, we became aware of characteristics that do not completely fit autosomal dominant inheritance pattern: (1) All the affected members invariably carry the mutation but there are some unaffected members who also carry the mutation. (2) Inheritance rate is closer to 25 % rather than 50 %. (3) The disease originated in mating of unaffected individuals. These fact prompted us to analyse another gene called *ROM1* for another gene defect that affect the phenotype of the patients with this mutation. We identified 2 distinct *null* mutations in *ROM1* gene in these families. These are both 1 base-pair insetion, termed Gly80(1-bp ins) and Leu114(1-bp ins). In the 3 families, only the double heterozygotes with both peripherin/*RDS* Leu185Pro and *ROM1 null* mutation show typical clinical picture of retinitis pigmentosa, and single heterozygote with either peripherin/*RDS* mutation alone or *ROM1 null* mutation alone do not develop disease. *ROM1* and periherin/*RDS* are homologous in terms of retina-specific expression, nucleotide and amino acid sequences. The protein product of these 2 genes are membrane-bound and reported to interact with each other. These facts and our results suggest that this is the first example of non-allelic non-complementation in human. In addition, this "digenic" inheritance gives the simplest paradigm of polygenic inherited disease in human.

A-35

Mucopolysaccharidosis IVA
Submicroscopic deletion of 16q24.3 in classical Morquio disease
Seiji FUKUDA, Shunji TOMATSU, Tatsuya OGAWA, Atushi YAMAGISHI, Kazuko SUKEGAWA, Tadao ORII (Dept. Pediatrics. Gifu Univ. Sch. of Med.) and Mitsuo MASUNO (Div. Med. Genet. Kanagawa Children's Medcal Center)
The N-acetylgalactosamine-6-sulfate sulfatase gene (GALNS), which is responsible for autosomal recessive Mucopolysaccharidosis IVA (MPSIVA), has been assigned to 16q24.3, where the adenine phosophoribosyltransferase (APRT) gene also localized. Molecular genetic studies on a severely affected patient with MPSIVA (Morquio disease) without karyotypic abnormality revealed a partial submicroscopic deletion of 16q24.3 and a single point mutation on the other allele, with no functional GALNS activity. The patient, his mother and siblings were hemizygous for GALNS and APRT loci, evidenced by informative RFLP and gene dosage analyses combined with a fluorescence *in situ* hybridization utilizing a partial genomic clone of GALNS, but heterozygosity was retained at the flanking markers. As estimated from the genetic distance between two flanking markers, size of the deletion was less than 3Mb. The remaining paternal allele encodes no GALNS activity on account of R386C mutation. Allelic loss of APRT is frequently observed in cancer tissues, thereby suggesting that the tumor suppressor gene locates near the APRT locus but no family member has evidence of any malignant disease and were phenotypically asymtomatic, despite this interstitial deletion of the Giemsa-light G band.

A-36

ANALYSIS OF THE HUMAN LYSOSOMAL ACID α-GLUCOSIDASE (GAA) GENE CAUSING ADULT-ONSET PATIENTS WITH ACID MALTASE DEFICIENCY.

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Acid maltase deficiency (AMD) is a rare metabolic disorder which is inherited as an autosomal recessive trait. We investigated GAA gene in 4 adult-onset AMD (case 1 and 2 are siblings, and case 3 and 4 are the patients from other families). Initially we checked the mutations which had previously described, there is no mutations except heterozygous mutation of nt. 2446 (exon 17) in case 1 and 2. Secondary, we studied using with SSCP (single strand conformation polymorphism) methods, suggested that there were 6 mutations in exon 3, 10, 12, 15, 17 and 20 in cases 1 and 2, 5 mutations in exon 4, 6, 10, 15 and 19 in case 3, and 4 mutations in exon 3, 4, 15, 20 in case 4, respectively. So we had sequenced these 6 exons in case 1. There are 3 mutations leading to amino acids alterations, a homozygous A^{596}-to G transition (His→Arg in exon 3), a heterozygous T^{1691}-to C transition (Phe→Ser in exon 12), and a heterozygous G^{2446}-to A transition (Val→Ile in exon 17). The G^{596} transition had previously described polymorphism for human GAA gene. So there is a possibility that, in cases 1 and 2, the compound heterozygous mutations in exon 12 and 17 cause adult-onset AMD.

A-37

Maternal anticipation of Dentatorubropallidoluysian atrophy (DRPLA) and somatic heterogeneity of CAG repeats

Masashi AOKI, Koji ABE, Takeshi KAMEYA, Ryo SAKUMA, Tetsuya NAGATA, Hiroshi KURODA, Sunao OKITA, Nobuyuki SATO, Koichi WATANABE, Muneshige TOBITA, Kenji TSUBURAYA (Dept. Neurology, Tohoku Univ., School of Med., Sendai), Kazuki HASHIMOTO (Dohoku National Hospital, Asahikawa), Mitsunori WATANABE, Masaki IKEDA (Dept. Neurology, Gunma Univ., Maebashi), Masahito OGASAWARA, Yoko AOKI, Shigeo KURE, Yoichi MATSUBARA, Kuniaki NARISAWA (Dept. Biochem. Genet., Tohoku Univ., Sendai) and Yasuto ITOYAMA (Dept. Neurology, Tohoku Univ., Sendai)

Recent studies have identified an unstable expansion of CAG repeat in a gene located on chromosome 12 as a cause of dentatoruburopallidoluysian atrophy (DRPLA). As the case with Huntington's disease or spinocerebellar ataxia type 1, almost all cases of severe DRPLA with juvenile onset are inherited from their father (paternal transmission) and had large expansions more than 60 repeats. We investigated two DRPLA families where anticipation is apparent by maternal transmission and offsprings show progressive myoclonic epilepsy with onset in childhood. Clinical anticipation by maternal transmission was also associated with the expanded allele size even though the degree of expansions seems to be smaller compared to the case with paternal transmissions. Moreover, the expanded allele showed multiple peaks, although subcloned expanded DNA was strict. This heterogeneity may also happen in heterogeneous in oocytes, and results in maternal anticipation even there is a dominant paternal bias of expansion.

A-38

CLINIACL FEATURES OF DENTATORUBRAL-PALLIDOLUYSIAN ATROPHY ARE CLOSELY RELATED TO UNSTABLE EXPANSIONS OF CAG REPEAT.

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Hereditary dentatorubral-pallidoluysian atrophy(DRPLA) is autosomal dominant neurologic disorder characterized by variable combinations of myoclonus,epilepsy,cerebellar ataxia,choreoathetosis, and dementia. By directly searching for cDNAs with CAG repeats as candidates,we found unstable expansion of a CAG repeat of CTG-B37 located on chromosome 12. There is a good correlation between the size of the CAG repeat expansion and the age of onset. Patients with progressive myoclonus epilepsy(PME) phenotype had larger expansions and earlier age of onset. Furthermore,most of the patients with PME phenotype inherited their expanded alleles from their affected fathers.

A-39

STRUCTURE AND EXPRESSION OF THE DRPLA GENE.

Shigeo NAGAFUCHI[1], Hiroko YANAGISAWA[1], Emiko OHSAKI[1,2], Keiko TADOKORO[1], Tadashi INOUE[2], Masao YAMADA[1] ([1]Natl. Children's Med. Res. Ctr., Tokyo, [2]Lab. Nucleic Acid Science, Nihon Univ., Kanagawa)

Dentatorubral and pallidoluysian atrophy (DRPLA) is a progressive neurodegenerative disorder associated with expansion of an unstable CAG repeat. We have isolated several overlapping cDNA clones and determined the nucleotide sequences of the DRPLA gene. The gene is ubiquitously expressed to form a single 4.5 kb transcript and encoded by an open reading frame of 1184 amino acids, in which a polyglutamin track with variable length starts at aa 484. Although the predicted amino acid sepuence does not reveal any function, it does contain several interesting motifs consisting of simple repeated amino acid sequences, two stretches of arginine-glutamic acid dipeptides.

A-40

GENOME ANALYSIS USING YAC CLONES SPANNING THE DENTATORUBRAL AND PALLIDOLUYSIAN ATROPHY (DRPLA) GENE. Takayuki TAKEDA[1,2], Shigeo NAGAFUCHI[1], Yutaka NAKAHORI[2], Yasuo NAKAGOME[2], Masao YAMADA[1] ([1]Natl. Children's Med. Res. Ctr., Tokyo, [2]Dept. Hum. Genet., Tokyo Univ., Tokyo)

We have shown that dentatorubral and pallidoluysian atrophy (DRPLA) is caused by expansion of triplet repeats of a gene on chromosome 12p (Nagafuchi et al. Nature Genet. 6, 14-18, 1994). The nucleotide sequence of the DRPLA gene has been determined as reported in this annual meeting by Nagafuchi et al. To determine genomic organization in a long range arround the DRPLA gene, we screened two human YAC libraries (CEPH 'A' & 'B') by PCR using primers generated based on the DRPLA sequence. Three YAC clones of about 400, 430 and 2200 kb sizes, respectively, have so far been identified as having the DRPLA gene. Restriction sites of rare-cutting enzymes were determined by partial digestion of the clones following hybridization with a DNA fragment derived from the vector arms. Constructed maps predicted that at least one of the clones were a chimera. Further characterization is now in progress by FISH and Alu PCR techniques.

A-41

GENOMIC ORGANIZATION OF THE HUMAN DRPLA GENE, WHICH HAS EXPANDED CAG REPEATS IN DENTATORUBRAL AND PALLIDOLUYSIAN ATROPHY. Emiko OHSAKI[1,2], Shigeo NAGAFUCHI[1], Hiroko YANAGISAWA[1], Tadashi INOUE[2], Masao YAMADA[1] ([1]Natl. Children's Med. Res. Ctr., Tokyo, [2]Lab. of Nucleic Acid Science, Nihon Univ., Kanagawa)

Dentatorubral and pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder characterized by combined systemic degeneration of the dentatofugal and pallidofugal pathways. We have shown that DRPLA is caused by expansion of triplet repeats of a gene on chromosome 12p (Nagafuchi et al. Nature Genet. 6, 14-18, 1994). The nucleotide sequence of cDNA of the gene has been determined as reported in this annual meeting by Nagafuchi et al. Here, we report a genomic organization of the gene. We have isolated lambda phage clones carrying the gene, made a restriction map, and determined exon-intron boundaries. Although analysis of the 5' end has not been completed, the DRPLA gene was consisted of at least 10 exons encompassing about 15 kb. The gene encoding small nuclear RNA U7 was localized about 1.3 kb downstream of the DRPLA gene.

A-42

A NOVEL GENE WITH A HIGH HOMOLOGY TO THE HUMAN DRPLA GENE.

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We have shown that dentatorubral and pallidoluysian atrophy (DRPLA) is caused by expansion of triplet repeats of a gene on chromosome 12p (Nagafuchi et al. Nature Genet. 6, 14-18, 1994). The nucleotide sequence of the DRPLA gene has been determined as reported in this annual meeting by Nagafuchi et al. The sequence had a homology to M78755, an expression sequence tag of the human brain reported by Adams et al. in Nature 355, 632-634, 1992. The reported M78755 sequence, consisted of only 395 bp, had a 64% homology to a C-terminal portion of the DRPLA sequence. Both sequences could encode an identical stretch of 21 amino acids although 15 out of 63 nucleotides differed. To characterize the gene represented by the M78755 sequence, we isolated several cDNA clones and determined nucleotide sequence. The homology of the amino acid sequences between the two genes could be extended considerablly in both directions from the core sequence. The C-terminal half of the molecules had a 60% homology and interesting motifs were well conserved.

A-43

MUTATIONAL ANALYSIS OF PATIENTS WITH VON HIPPEL-LINDAU DISEASE IN JAPANESE POPULATIONS.

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Von Hippel-Lindau (VHL) disease is an autosomal dominantly inherited disorder characterized by a predisposition to develop hemangioblastomas of the central nervous system and retina, pheochromocytomas and renal cell carcinomas. The VHL tumor suppressor gene has recently been clond by positional cloning. We analysed 32 Japanese patients with VHL for germ-line mutations by PCR-SSCP and conventional Southern analysis. We found 16 mutations including 7 missense, 6 deletion and 1 insertion mutations by PCR-SSCP and DNA sequencing, and also found aberrant bands in 3 cases by Southen analysis. Thus, germ-line mutations were detected in total 19 out of 32 (59%) patients tested and in their families, accurate presymtomatic diagnosis was feasible. Among these families, 2 had pheochromocytomas as part of VHL manifestation. One family had a germ-line mutation at codon 238 (nucleotide 712: C to T), that was previously reported as one of the pheo(+) 'hotspot' in the white race, whereas the other had an unique mutation; 20 base-pair insertion at nt. 776 in exon 3. Mutation detections will facilitate VHL genotype-phenotype correlations and may provide some clues to functional analysis of VHL prtein as a tumor suppressor.

A-44

SINGLE AMINO ACID SUBSTITUTION AFFECTING TRANSPORTING OF ORNITHINE AMINOTRANSFERASE PRECURSOR PROTEIN TO MITOCHONDRIA.
Tatsuhiko KOBAYASHI, Hisamitsu OGAWA[1], Masao KASAHARA[2], Zenji SHIOZAWA[3] and Takeo MATSUZAWA (Dept. Biochem., [1]Biol. and [2]Pathol., Fujita Health Univ. Sch. Med., Aichi, [3]Dept. Int. Med., Yamanashi Med. College, Yamanashi)

The molecular basis of ornithine aminotransferase (OAT) deficiency, gyrate atrophy of the choroid and retina (GACR), was studied. Lymphocytes from a GACR patient contained less than 10% of OAT activity compared with a normal subject. Nucleotide sequence analysis of the amplified OAT cDNA from the patient's lymphocytes mRNA by RT-PCR method revealed a single base change from C to G, resulting in substitution of glutamine with glutamic acid at position 90 in the mature OAT protein. Western blot analysis of the mutant OAT expressed in CHO cells indicated that the patient's OAT should be degraded rapidly after synthesis. Western blot analysis and immunoelectron microscopic observation of the mutant OAT expressed in a baculovirus expression system elucidated that the mutant OAT protein accumulated on cytosolic polysomes, outside mitochondria, as precursor protein of 49 kDa. These results suggest that substitution of the glutamine-90 with glutamic acid results in reduction of OAT activity because of the mutant OAT precursor from the GACR patient can not be transported to mitochondria.

A-45

MOLECULAR BASIS OF MUCOPOLYSACCHARIDOSIS TYPE ⅣA.
Naoto YAMADA, Shunji TOMATSU, Seiji FUKUDA, Kouji ISOGAI, Zenichirou KATOU, Tatuya OGAWA, Kazuko SUKEGAWA, Naomi KONDOU and Tadao ORII
(Department of Pediatrics, Gifu University School of Medicine, Gifu)

ムコ多糖症ⅣA型は通称Morquio disese とも呼ばれN-acetylgalactosamine-6-sulfate sulfatase (以下GALNSと略す)の異常による常染色体劣性の遺伝性疾患である。
臨床症状として高度の軟骨内骨化障害による骨変形、低身長、角膜混濁、ケラタン硫酸・コンドロイチン6硫酸尿などを認めるが、知能障害は認められない疾患である。
演者らは既に欠損酵素GALANSの精製、抗体の調製，全長cDNAのクローニング、ゲノム遺伝子の構造，病因と考えられるエクソンの遺伝子変異部位を同定し報告した。今回さらに症例を重ねPCR-SSCP法を用いて欧米人・日本人の各患者より得られたPCR断片につき遺伝子変異スクリーニングを行い新たなエクソン内に遺伝子変異部位を同定したのでここに報告する。この方法で総計41個の変異が認められた。

- 本疾患の病因遺伝子変異部位非常に多様性に富んでいた。
- 欧米人でのcommon mutationはI113Fであり、日本人でのcommon mutationはdouble gene delitionがであった。
- 日本人、欧米人とも共通な変異部位がR386C, P151Lの２つのみであり、その他の変異は全く共通しておらず本疾患において遺伝子変異における人種差認められました。

A-46

MOLECULAR ANALYSIS OF DIHYDROPTERIDINE REDUCTASE DEFICIENCY.
Hiroyuki IKEDA, Yoichi MATSUBARA, Kuniaki NARISAWA (Department of Biochemical Genetics, Tohoku University School of Medicine, Sendai)

Inborn errors of dihydropteridine reductase (DHPR) result in hyperphenylalaninemia and deficiency of various neurotransmitters in central nervous system, causing severe neurological symptoms. We studied three Japanese patients with DHPR deficiency. DHPR mRNA was expressed normally in case 1, but markedly decreased in case 2 and 3. A missense mutation (Trp36-to-Arg) was identified in case 1 in homozygous form. The mutation was found to abolish DHPR activity according to *in vitro* expression study using COS-7 cells. RT-PCR of DHPR mRNA from case 2 produced a DNA fragment with 152 bp-insertion. Analysis of genomic DNA indicated that the insertion was derived from intron 3 of the DHPR gene and an intronic A-to-G substitution was present adjacent to the inserted sequence. The inserted sequence contained a termination codon, which is likely to affect the stability of mRNA. The nucleotide change generated a sequence similar to RNA splicing donor site and probably activated a upstream cryptic accepter site, thus producing a 'new' exon in intron 3. Amplified DHPR cDNA from case 3 did not contain any nucleotide change, suggesting that a mutation may be present in non-coding region or an intron and affects the expression or stability of mRNA.

A-47

POLYMORPHIC GENETIC ANALYSIS OF JAPANESE FAMILIES WITH PHENYL-KETONURIA BY SHORT TANDEM REPEAT SYSTEM.
Youngbo KANG (Dept. of Pediatr., National Sengokusou Hospital, Kaizuka), Yoshiyuki OKANO, Gen ISSHIKI (Dept. of Pediatr., Osaka City Univ. Med. School, Osaka), Yutaka HASE (Osaka City Turumi Public Health Center) and Toshiaki OURA,(Osaka Municipal Rehabilitation Center for The Disabled, Osaka)

Phenylketonuria (PKU) is an autosomal recessive disease caused by a deficiency of phenylalanine hydroxylase (PAH), which causes severe mental retardation unless the child is maintained on a strict low-phenylalanine diet. As PAH protein is expressed mainly in human liver and not in amniotic cells and chorionic villi, prenatal diagnosis in PKU has requested the genomic DNA analysis. The short tandem repeat (STR) system, which is located in intron 3, has heterogeneity of 75% in Chinese and 80% in European Caucasian populations. We report here the heterogeneity of STR in the Japanese population. Allele frequencies were identified through 19 PKU families in Japan. The size of STR ranged from 228 bp to 252 bp. The 244 bp was the most prevalent in normal (39%) and PKU chromosomes (50%). The 240 bp was the next prevalent in normal (21%) and PKU chromosomes (12.4%). The pattern of STR showed high degree of polymorphism. There was not a statiscally significant difference in the distribution of these STR alleles among normal and mutant chromosomes by the χ square test. This STR system showed the average level of heterozygosity of 70% in Japanese population, therefore the probability of genetic diagnosis was 70%. Actually, 15 of 19 PKU families in Japanese allowed the prenatal diagnosis by the STR system. The high degree of polymorphism and strong Mendelian segregation of the STR system is useful for prenatal diagnosis in Japanese PKU families.

A-48

MISSENSE MUTATIONS OF CARDIAC MYOSIN HEAVY-CHAIN GENE IN HYPERTROPHIC CARDIOMYOPATHY DIFFER ACCORDING TO RACE.
Shoichi ARAI (Suzuka University of Medical Science and Technology, Mie) and Misa KIMURA, Shin-ichiro IMAMURA, Atsuyoshi TAKAO, Kazuo MOMMA, Saichi HOSODA, Rumiko MATSUOKA (Tokyo Women's Medical College, Tokyo) and Kouzin KAMINO (Osaka University,Osaka) and Kunitaka JOH-O (Kyushu Welfare Pension Hospital, Fukuoka).

Seven familial hypertrophic cardiomyopathy (HCM) families and 50 unrelated healthy controls were examined a linkage study to catdiac myosin heavy chain (MHC). The results showed that one familial HCM family had recombinant DNA and the lod score of 6 familial HCM families were between 0-1.4. The genetic analysis in the β-MHC gene revealed one missense mutation (741 Gly→Trp) in one family (4 members with HCM). Also, mutations were found in the exon 35 of α-MHC (1658 Asp→Asp) resulting in FokI site polymorphism and in the intron 34, and in the intron 14 of the β-MHC in Noonan syndrome in 3 sporadic cases. Thirty missense mutations of the β-MHC gene in 49 families have been reported world-wide. Only one mutation was found in both Caucasian and Japanese familes.

A-49

DELETIONS OF THE ELASTIN GENE IN WILLIAMS SYNDROME. Yuji MORIMOTO, Akira KUWANO, Ikuko KONDO(Dept Hyg, Ehime Univ, Ehime) Katsuko KUWAJIMA(Ibaraki Pref. Handicap Children's Hosp, Ibaraki), Yoshimitsu FUKUSHIMA(Saitama Children's Med. Ctr, Saitama), Masato TSUKAHARA(Dept Pediatr. Yamaguchi Univ, Yamaguchi), Hitoshi GANAHA(Okinawa chubu Hosp, Okinawa), Tetsuro KAMIYA(Dept. Pediatr. National Cardiovascular Ctr, Osaka), Kiyotaka TOMIWA(Osaka Municipal Med Ctr) , Hidefumi TONOKI(Dept Pediatr. Hokkaido Univ, Hokkaido),

We screened 20 Japanese patients with Williams syndrome (WS) for the deletions in ELN both by polymerase chain reaction (PCR) and/or fluorescence *in situ* hybridization (FISH). Overall, 19 of 20 patients demonstrated a detetion of ELN. By using PCR, eight patients were informative, four patients had maternal, and remaining four had paternal deletions, indicated that imprinting genes might not be involved. Only one patient with no deletions of ELN showed typical WS phenotype but normal intelligence and no hoarseness. This patient will be important in delineating the critical regions responsible for mental retardation.

A-50

MOLECULAR ANALYSIS OF JAPANESE PATIENTS WITH HOLOCARBOXYLASE SYNTHETASE DEFICIENCY.

Yoko AOKI, Yoichi SUZUKI, Osamu SAKAMOTO, Xue LI and Kuniaki NARISAWA
(Department of Biochemical Genetics, Tohoku University School of Medicine, Sendai)

Holocarboxylase synthetase (HCS) deficiency is an autosomal recessive disorder characterized by combined organic aciduria, metabolic ketoacidosis, and dermatitis. These clinical symptoms are dramatically improved by administration of biotin. To analyze the molecular basis of the disease, we have recently isolated human HCScDNA clones. We studied one Japanese sib case and found two distinctive mutations, one base transition (T997C) and deletion of a G residue at base position 1067 by analyzing HCS mRNA. The genomic analysis confirmed the compound heterozygosity of the affected sibs. The T997C mutation causes amino acid substitution of Pro^{237} for Leu^{237}, while one base deletion results in a frame shift followed by a premature termination. We analyzed three additional unrelated Japanese patients by allele specific oligonucleotide hybridization analysis and detected one base deletion in one patient at heterozygous form. We also detected the T997C mutation in three patients; two patients were heterozygotes and one patient was a homozygote. The T997C mutation was not detected in 108 normal healthy Japanese children (216 alleles). A transient expression study showed that the T997C mutation was responsible for decreased HCS activity. These results suggested that the T997C is a frequent mutation among Japanese patients with HCS deficiency.

A-51

GENETIC ANALYSIS OF MILD-FORM MAPLE SYRUP URINE DISEASE (MSUD).

Yumi HAYASHIDA, Motoko TSURUTA, Hiroshi MITSUBUCHI, Yasuhiro INDO, Kohji OHTA, Tomoyasu KAWANO, Md. Azharul KARIM, Fumio ENDO, Yoshitaka NOBUKUNI, Ichiro MATSUDA (Dept. Pediatrics, Kumamoto University School of Medicine, Kumamoto, Japan)

MSUD is an autosomal recessive inborn error of metabolism caused by dysfunction of any of the subunits (E1α, E1β and E2) of the branched-chain α-keto acid dehydrogenase complex (BCKDH). We analyzed three MSUD patients with the mild clinical phenotype, detected by neonatal screening. BCKDH activities of these patients were significantly higher than those of classical patients. Both E1α and E1β subunits were seen to be reduced by the immunoblot analysis, although a similar amount of E2 subunit, as control, existed in all cells. Five novel mutations were identified in the E1α gene, 4 were missense mutations and the other was a 3 base deletion, causing substitution and deletion, without generating frameshift downstream. These mutations are I 168 T / L 382 P& A 383 del in KM11, R 122 Q / R 318 W in KM12, and Y 121 C / Y 121 C in KM13. A patient (KM 13) and others (KM 11 and 12) were homozygote and compound-heterozygotes, respectively. At least one mutant allele of each patient was located in exon 5 of the E1α gene. Thus some missense mutation(s) in exon 5 of the E1α gene may be related to mild phenotype MSUD.

A−52

DEFICIENCY OF THE E2 SUBUNIT IN THE BRANCHED-CHAIN α-KETO ACID DEHYDROGENASE (BCKDH) DUE TO A SINGLE BASE SUBSTITUTION OF INTRON 8 , RESULTING IN INSERTION OF AN INTRONIC SEQUENCE INTO THE mRNA IN A GIRL WITH MAPLE SYRUP URINE DISEASE(MSUD).

Hiroshi MITSUBUCHI, Yumi HAYASHIDA, Motoko TSURUTA, Yasuhiro INDO, Kohji OHTA, Tomoyasu KAWANO, Md.Azuharul KARIM, Fumio ENDO, Ichiro MATSUDA (Dept. Pediatrics, Kumamoto University School of Medicine, Kumamoto)

KM 03, a girl born to consanguineous parents was diagnosed as a case of MSUD during neonatal screening. The BCKDH activity of the patient's cells was 6.4% of the control. Immunoblot analysis of the lymphoblastoid cells derived from this patient lacked the E2 protein of the BCKDH complex. To search for mutations within the coding sequence for BCKDH-E2 cDNA, RNA was isolated from the cells and used to synthesize cDNAs. This material was subjected to PCR amplification using a set of primers. Two cDNAs, including a normal-sized one and a long one, were amplified. To characterize the mutation, the long cDNA fragment as well as a normal-sized one were subcloned and sequenced. A 125 bp of unknown sequence was inserted into the junction of exons 8 and 9 in the long cDNA fragment, causing a premature termination downstream. No changes were apparent in the normal size cDNA. Analysis of the patient's genomic DNA revealed that the inserted DNA fragment was part of intron 8 and was recognized as a new exon due to a single base substitution (A to G), in the same intron. This substitution yielded a new 5'-splice donor site in intron 8 and probably activated a cryptic splice site located at the 5'-end of the new exon. This type of mutation is rare in that it was identified in only one patient with thalassemia. To our knowledge, this is the first example of formation of a new exon due to a single base substitution in an intron and causing an inborn error of metabolism.

A−53

MISSENCE MUTATIONS OF 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE DEFICIENCY IN JAPANESE.

Takuji IMAMURA, Yoshiyuki OKANO, Haruo SHINTAKU, Gen ISSHIKI (Dept. of Pediatr., Osaka City University Medical School, Osaka), Yutaka HASE (Osaka City Turumi Public Health Center), Toshiaki OURA (Osaka Municipal Rehabilitation Center for The Disabled, Osaka)

Tetrahydrobiopterin (BH_4) is a cofactor of phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase. BH_4 deficiency impairs the biosynthesis of neurotransmitters. Patients with BH_4 deficiency will go on to develop progressive neurologic deterioration. We identified human PTPS cDNA and found mutations of 6-pyruvoyl-tetrahydropterin synthase (PTPS) gene in three Japanese patients with PTPS deficiency. Parents of two patients were unrelated and parent of one patient were second cousin.

The rat PTPS cDNA cloned in pUC18 was labelled with ^{32}P, and used for screening a λ gt11-vector-derived human fibroblast cDNA library. The sequence of human PTPS cDNA was the same as the published sequence in amino acid coding region. Total RNA was isolated from lymphoblastoid cells transformed with Epstein-Barr virus by acid thiocyanate guanidine method. PTPS cDNA was amplified from the total RNA by RT-PCR method, and was directly sequenced using Dynabeads™ (Dynal co. ltd.).

One missense mutation was a C to T transition in the PTPS gene, resulting in the substitution of Pro by Ser at the codon 87 (P87S). Another missense mutation was a G to A transition in the PTPS gene, resulting in the substitution of Asp by Asn at the codon 96 (D96N). This P87S mutation was found as homozygote in two patients and P87S/D96N mutations were found as compound heterozygote in one patient. Patients with P87S/P87S mutations has 0% of PTPS activity in erythrocytes and Patient with P87S/D96N mutations has 6% of PTPS activity in erythrocytes. From these results, the different genotype show the different PTPS activity in erythrocytes, P87S mutation reduced the enzyme activity than D96N mutation. These P87S mutation and D96N mutation are found to be a conserved region in the homologous enzyme from salmon, rat and human, and is an important region of PTPS activity.

A-54

MOLECULAR BASIS OF CARNITINE PALMITOYLTRANSFERASE(CPT) I DEFICIENCY: cDNA CLONING OF HUMAN CPT I AND MOLECULAR PATHOLOGY OF TWO JAPANESE CASES PRESENTING REYE-LIKE EPISODES.

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Three cDNA clones encoding the human liver-type carnitine palmitoyltransferase(CPT) I were isolated. The human CPT I had 87% identity with the rat enzyme, and predicted Mr. was 88,218Da. Two Japanese patients of CPT I deficiency presenting Reye-like episodes were analyzed with these clones. RNA blot analysis, and reverse transcription, amplification, and sequencing of the CPT I transcript identified the possibility of the splicing mutation in one patient, and a single T to G transversion at nucleotide 96 that predicted nonsense mutation in residue 36 in the other.

A-55

A KERATIN K14 GENE MUTATION IN A PATIENT WITHDOWLING-MEARA TYPE OF EPIDERMOLYSIS BULLOSA.

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Epidermolysis bullosa simplex (EBS) is caused by an aberration of the keratin intermediate filaments and recent studies indicated causal mutations in the keratin K14 and K5 genes. In this study, we examined keratin K14/5 gene mutation in a Japanese patient with EBS Dowling-Meara (EBSDM). The patient had a C to transition at the first position of con 125, which resulted in Arg →Cys at the N terminus of the rod domain in the keratin K14 gene. The mutation position described here was identical to those reported in some other EBSDM patients. Our result revealed mutation in the helix initiation peptide of keratin K14 and, together with the results of other workers, suggests that the mutation in the keratin K14 gene of EBSDM sufferers occurs in virtually every ethnic group and geographical area.

A-56

A NOVEL DETECTION METHOD FOR THE K-VARIANT OF BUTYRYLCHOLINESTERASE BASED ON PCR PRIMER INTRODUCED RESTRICTION ANALYSIS. Kenji SHIBUTA, Masako ABE, and Tomokazu SUZUKI (Dept. Clin. Genet., Med. Inst. Bioregulation, Kyushu Univ., Beppu)

Human butyrylcholinesterase (EC. 3.1.1.8; BChE) is encoded by a single gene (BCHE) which is composed of four exons. Several genetic variants have been shown to cause prolonged apnea in patients who were given a standard amount of the muscle relaxant drug succinylcholine. The K-variant was found to cause a 33% reduction in BChE activity by a point mutation at nucleotide position 1615 (GCA/ACA) in exon 4 of BCHE, which might cause the Ala 539 to Thr. This mutation neither creates nor destroys any restriction site. Therefore, in an attempt to detect the K-variant both reliably and rapidly, we developed a two step method based on PCR primer introduced restriction analysis (PCR-PIRA). The first step was used to introduce a new *Fnu*4HI site into the normal allele for screening test, while the second step was performed to create a new *Mae*III site on the variant allele for a specific test. This method thus enabled us to distinguish clearly the K-variant from the normal allele, and also showed that the frequency of the K-variant allele is 0.164 in the Japanese population, which was similar to that of whites.

A-57

MUTATIONS RESPONSIBLE FOR HUMAN ERYTHROCYTE AMP DEAMINASE DEFICIENCY. Nobuaki OGASAWARA, Yasukazu YAMADA, Haruko GOTO (Dept. Genet., Inst. Developmental Res, Aichi Prefectural Colony, Aichi)

Erythrocyte specific AMP deaminase deficiency identified firstly by us, is clinically completely asymptomatic. The inheritance is autosomal recessive and the heterozygote frequency is estimated at about 1/30.

A point mutation (C1717T) identified on the human erythrocyte AMP deaminase gene (*AMPD-3*) causes the enzyme deficiency (*Hum. Mol. Genet.* 3, 331-334). As the C1717T seems to be very frequent at least in Japanese, we screened mutant genes. After screening of 2,600 Japanese blood samples, 61 had about a half the enzyme activity of control and 2 had none. C1717T was detected in both alleles of 2 individuals with complete deficiency and in one allele of the 45 individuals with partial deficiency, but not in 16 of the latter. Sequencing of these 16 samples diagnosed as heterozygous revealed 9 heterogeneous mutations in one allele of 12 individuals. Three mutations (T600D, T971C, and C991T) were detected by RT-PCR using B-lymphoblast cell lines and 6 other mutations (C930G, C959T, C1204T, C1300T, T1348A, and C1754T) were found by analyzing the genomic DNAs. However, mutations in the remaining 4 samples were not found. Thus, the human erythrocyte AMP deaminase deficiency in Japanese is associated with 75 % of the major mutation (C1717T) and 25 % of other heterogeneous mutations.

A-58

HYPOALPHALIPOPROTEINEMIA DUE TO MUTATIONS OF APOLIPOPROTEIN A-I GENE. Kimiko KOBAYASHI, Hisako YANAGI, Hiromi FUKAYAMA, Yae SHIMAKURA*, and Hideo HAMAGUCHI (Dept. Med. Genet., Inst. Basic Med. Sci., and *Dept. Pediatr., Univ. Tsukuba, Tsukuba)

Low plasma concentrations of high-density lipoprotein (HDL) cholesterol as well as its major protein component, apolipoprotein A-I (apo A-I), are the major risk factors of coronary heart disease, and exhibit significant familial aggregation. To investigate the frequency of the hypoalphalipoproteinemia due to the mutant apo A-I gene, we have analyzed the sequences of all exons, splice junctions and promoter region of apo A-I gene from six pupils with low HDL-cholesterol (<35 mg/dl) and apo A-I (<80 mg/dl) levels, who have been screened from 1068 apparently healthy school children in a school survey. We have detected two kinds of frameshift mutations from them. These two frameshift mutations were not observed 70 other pupils with low HDL-cholesterol levels (<40 mg/dl) detected in the school survey. The findings suggest that the hypoalphalipoproteinemia due to the mutant apo A-I gene is a relatively common genetic disorder that affects about one in 500 peoples. Family studies could be done in one of the children with the apo A-I gene mutation. Family data showed that the mutation causes autosomal dominant hypoalphalipoproteinemia and results in the gene dosage effects on HDL cholesterol and apo A-I levels.

A-59

ANALYSIS OF APOLIPOPROTEIN E VARIANT GENE : E5s ($_{171}$Gly→Lys).
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Apolipoprotien E (apo E) plays an important role in lipoprotein catabolism. It has been shown that a part of apo E variants lead to hyperlipidemia. Assessment of apo E phenotype by two-dimensional gel electrophoresis (Two-DE) of 819 Japanese males who visited a health care center in Tokyo, identified a rare apo E phenotype (E4/5s). His lipid levels T. cho, TG, apo B and apo E were 225mg/dl, 81mg/dl, 106mg/dl, 5.8mg/dl, respectively. Two-DE pattern showed that the PI of E5s was two units more basic than that of the common type E3. In order to characterize the E5s, we have studied its DNA sequence by T-vector cloning after PCR amplification and an automated sequencing. A G→A substitution converted glutamic acid (GAG) at position 171 of mature protein to Lysine (AAG). Thereby it led to a PI shift with 2 positive charge units to E3 protein and producing the apo E5s variant.

A-60

INDIRECT DNA DIAGNOSIS OF PKD1 USING MICROSATELLITE MARKERS
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Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder that frequently results in renal failure due to progressive cyst development. The prevalence is approximately 1 in 1000 individuals among all races. At least two loci have been reported: one is mapped to 16p13.3 and the other mapped to 4q13-q23.
In this study, we attempted to established an indirect DNA diagnosis by PCR using microsatellite markers and buccal cell DNA. Approximately 5μg of DNAs were isolated from 100 streaks of cheeks by successive treatment of proteinaseK, phenol, chloroform and ethanol. Conditions for PCR were established for six different satellite markers including SM7, 16AC2.5, SM5B, MS7, KG8 and SM6. Markers SM7 and 16AC2.5 revealed clear polymorphisms among Japanese and were most informative. This simple method is being used for the patients' diagnosis.

A-61

DETECTION OF HETEROZYGOUS GENE DELETION IN DUCHENNE/BECKER MUSCULAR DYSTROPHY CARRIER BY THE POLYMERASE CHAIN REACTION. Ryoji HIRAMATSU, Masako ABE, Tomokazu SUZUKI (Dept. Clin. Genetics, Med. Inst. Bioregulation, Kyushu Univ., Beppu)

Gene dosage has to be determined to detect heterozygous deletions of dystrophin gene in female carriers. We now report quantitative method for dystrophin gene (*DYS*) by PCR using N-acetyltransferase gene (*NAT2*) as an internal standard. Radioactivity of PCR products was analyzed on 2.0-3.0% Agarose gels using BAS1000 (Fuji Photo Film). Gene dosage was calculated by comparing *DYS* exon/*NAT2* ratios in test samples with those in 6 control females. Gene dosage for control males, a definite carrier, and another possible carrier was 44±1 % (n=4), 37%, and 54%, respectively. Gene dosage for a non-carrier female diagnosed by Southern blot analysis previously was 104% when determined by our method. The present method is a rapid and easy technique for detecting carriers of dystrophin gene deletions.

A-63

IMPRINTING OF THE WT1 GENE: IMPRINTING POLYMORPHISM AND TISSUE-SPECIFIC MONOALLELIC EXPRESSION

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We previously reported that the human Wilms tumor suppressor gene (*WT1*) is monoallelically expressed in the placenta and fetal brain and that the imprinting pattern (maternal or biparental expression) differs in different individuals. In order to confirm the imprinting polymorphism and tissue specificity, we examined allele-specific expression of *WT1* in 46 chorionic villus samples of 5-22 gestational weeks and in 4 visceral organs (liver, lung, heart, intestine) from one 21-week-old fetus, using a CA repeat and a *Hin*fI RFLP both within 3'-UTR of *WT1* as markers. Of 18 informative villus samples, 11 (61%) expressed *WT1* biallelically, while the expression in the remaining 7 were monoallelic. In the 4 organs, *WT1* was expressed biallelically, although the expression in the villus tissue from the same fetus was monoallelic. As there was no correlation between gestational week and monoallelic expression, it is concluded that different patterns of allelic expression of *WT1* in early human development is polymorphic and tissue-specific.

A-64

ISOLATION AND MAPPING OF HUMAN HOMOLOGUES OF A MOUSE IMPRINTED GENE *U2af1-rs1* .

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We have isolated human homologues of the mouse imprinted gene, *U2af1-rs1* . Two different types of cDNAs and three distinct genomic DNAs belonging to different groups were isolated. We have specified chromosomal genes corresponding to each cDNA by restriction mapping and sequencing. As the result of FISH analysis, group 1 gene (corresponding to type 1 cDNA) and group 2 gene (corresponding to type 2 cDNA) were mapped on chromosome 5q22 and chromosome Xp22.1, respectively. We designated group1 and group2 gene as human *U2af1-rs1* and *U2af1-rs2* , respectively, because these genes corresponded to mouse *U2af1-rs1* (chromosome 11) and *U2af1-rs2* (chromosome X), which we had isolated and mapped (Hatada et al., 1993). Amino acid sequences of human *U2af1-rs1* and *-rs2* showed a significant homology to U2AF small subunits. Group3 gene, designated as *U2af1-rs3*, of which cDNA hasn't yet been isolated, was mapped on chromosome 19p 13.2.

A-65

ISOLATION OF PROMOTER SEQUENCE OF GLYCOPROTEIN D USING INVERTED PCR METHOD. Sadahiko IWAMOTO, Toshinori OMI, Eiji KAJII and Sigenori IKEMOTO (Dept. Legal Med. and Human Genet., Jichi Medical School, Tochigi)

Glycoprotein D (gpD) cDNA which associate with Duffy blood group antigen has been cloned by Chaudhuri et al (1993). We report the isolation of promoter sequence of gpD gene using inverted PCR method and the base changes observed in the sequence from Duffy negative individuals. By a Southern blotting analysis probed with gpD cDNA, gpD gene was revealed to be composed of three DNA fragments; 1.1kb SacI, 1.9kb EcoRI and their intervening 47bp fragments. We cloned the 1.1kb SacI fragment by inverted PCR procedure. The 5' flanking region that extend upstream to -899bp from transcription start site was contained in the IPCR product. Wile no TATA or CCAAT boxes are present in the promoter sequence, several transcription factor binding motifs are contained, including AP-1, HNF-5, TCF-1, ApoE B2, W-element, H-APF-1 and Sp-1. Two Duffy negative individuals showed respective base changes; T→C substitution at -365 and C→T substitution at -128, respectively. These information will contribute to the understanding of how this promoter sequence regulates the transcription of Duffy mRNA.

A-66

A MODEL FOR ALTERNATIVE SPLICING: INTERPLAY BETWEEN NEGATIVE AND POSITIVE CONTROL ELEMENTS. Takayuki MORISAKI, Hiroko MORISAKI and Edward W. HOLMES (Department of Medicine and Genetics, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.)

Alternative splicing is a fundamental mechanism for expanding gene diversity - commonly used in myocytes. We have used AMPD1 as a model gene to identify cis-acting sequences that control alternative splicing. Results may be clinically relevant because of the potential rescue of AMPD deficiency through alternative splicing. Prior studies have established that Exon 2 is intrinsically defective and difficult to recognize. In the oresent study, the AMPD1 minigene has been subjected to an extensive mutational analysis and analyzed through transfection studies in myocytes and nonmyocyte cell lineages. The results demonstrate the molecular basis for masking of Exon 2 and identify elements essential for unmasking of this exon. On the negetive side, there are three defects which contribute to masking of Exon 2, i.e., a suboptimal 3' acceptor site, a suboptimal 5' donor splice site and close proximity of these sites to one another; improvement in anyone of these defects relieves masking. On the positive side, an exon recognition element (ERE), in the downstream intron facilitates recognition of this defective exon. ERE has at least three functional components. The decision to retain or exclude Exon 2 from mature transcripts appears to be determined by the interaction between these opposing forces.

A-67

DEFECTS IN MITOCHONDRIAL FUNCTIONS SEEN IN CYBRIDS HAVING MELAS-3243 or -3271 MUTATION.
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An A to G transition at nucleotide 3243 or a T to C at 3271 in the mitochondrial $tRNA^{Leu(UUR)}$ gene has been associated with MELAS, a maternally-inherited mitochondrial disorder. We transferred mitochondria harboring one of these mutations into a human cells lacking endogenous mtDNA (ρo cell), and analyzed to understand the genotype-phenotype relationship. Cybrids, containing >93% of the mutant genomes, showed : 1) decreased rate of synthesis and of steady-state levels of mitochondrial translation products, 2) reduced respiratory chain functions and 3) increased amounts of a novel unprocessed RNA species (RNA 19). Overall effects on mitochondrial functions are more severe in MELAS-3243 than that in MELAS-3271. Increased levels of RNA 19 were also recognized in biopsied muscles from 4 MELAS patients. Above date, combined with our previous date, suggesting that RNA 19 may play an important role in the pathogenesis of this mitochondrial diseases.

A-68

ESTABLISHMENT OF CHINES HAMSTER OVARY CELL LINES WITH REDUCED EXPRESSION OF GLUTATHIONE REDUCTASE AFTER ANTISENSE-ORIENTED GENE TRANSFECTION AND ASSESSMENT OF THE SENSITIVITY TO OXIDANT INJURY.
Hidefumi TONOKI (Dept. Pediatr., Hokkaido Univ. School of Med., Sapporo)

Glutathione reductase (GR) protects tissues from oxidant injury by catalysing the reduction of glutathione disulfiide (GSSG) to glutathione (GSH). In order to study the effect of GR deficiency in protecting cells from oxidant injury, we generated Chinese hamster ovary (CHO) cell lines stably transformed after antisense-oriented gene transfection. The coding region of the human GR was cloned using reverse transcription PCR method and then, ligated into MEP4 expression vector in an antisense orientation to the human metallothionein promoter and transfected to CHO cells with polybrene. Among 12 cell lines isolated, G17 showed to have the least GR activity (48% of the control), while another four were mildly GR deficient. Southern hybridization of genomic DNA digests revealed that the promoter-antisense coding region component was integrated. Northern hybridization detected reduced amount of GR transcript but no antisense message. Baseline GSH concentrations were lower in G17 than in control (25.7 ± 2.5 vs 36.1 ± 1.9 nmole/mg protein, $P<0.05$), while GSSG concentrations were higher (0.61 ± 0.19 vs 0.39 ± 0.09 nmole/mg protein, $P<0.05$). Four hours of exposure to 10 mM t-BuOOH resulted in greater LDH release in G17 than in control (57.3 ± 4.7 vs 32.1 ± 6.5 %, $P<0.05$). Similarly, G17 cells released more of their LDH to the media than did CHO cells in response to exposure to 95% O_2 for 72 hours (19.3 ± 5.0 vs 11.9 ± 5.4, $P<0.05$). The partial GR deficiency in G17 cells impairs their ability to recycle GSSG and this deficiency offers the best explanation for the increased sensitivity of these cells to injury by t-BuOOH or hyperoxia.

A-69

TRANSDUCTION OF HUMAN HEMATOPOIETIC PRECURSORS BY VIRAL VECTORS.

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We examined the effectiveness of viral vectors (retrovirus and adenovirus) for gene transfer of adenosine deaminase (ADA) into human bone marrow (BM). Mononuclear BM cells from normal allogeneic donors were infected by co-cultivation with the amphotropic retrovirus producing cell line in the presence of IL-3 and IL-6. The infection efficiency of clonogenic progenitors was 50-90%. Using myeloid long term culture (LTC), exogenous ADA transcripts were detected in 20% of total colonies at 6 weeks post-infection. The transduced ADA activity was analyzed by a microradioassay and was 2.0 fold higher than controls at week 9. These results suggest that gene transfer into primitive hematopoietic progenitors related to the pluripotent stem cells has been achieved. Mononuclear cells from one ADA-deficient and two normal human BM samples were infected by an adenoviral vector and kept in LTC. In two out of four experiments, the ADA activity decreased with passage, probably because integration of adenovirus into the host genome is very rare. Unexpectedly, sustained expression from Ad-ADA was observed in the other two experiments. At the end of the experiments (8-11 weeks post-infection), culture medium was harvested to measure infectious virus. Only from BM cultures which showed sustained expression of ADA, free Ad-ADA virus was detected. These results might raise a concern regarding the safety aspect of adenovirus vectors as a tool for human gene therapy.

A-70

RETROVIRUS-MEDIATED RECONSTITUTION OF RESPIRATORY BURST ACTIVITY IN X-LINKED CHRONIC GRANULOMATOUS DISEASE CELLS.

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Chronic granulomatous disease (CGD) is a recessive disorder in which blood phagocytes cannot generate superoxide and derivative microbicidal oxidants. Affected patients suffer recurrent and often life-threatening infections. CGD results from mutations in the respiratory burst oxidase complex, with two thirds of cases associated with defects in the X-linked gene for gp91-*phox* (X-CGD). We prepared a recombinant retrovirus (AmZip/PGK-gp91) to infect a human myelomonocytic X-CGD cell line (X-CGD PLB) and murine primary bone marrow cells. In the present study, 15% of X-CGD PLB cells were functionally corrected, with reconstitution of up to 50% of wild type activity. When murine primary bone marrow cells were infected and transfused into lethally irradiated mice, provirus integration was detected in 17% of the day 12 spleen colonies (CFU-S). These studies suggest the feasibility of somatic gene therapy of X-CGD using AmZip/PGK-gp91 or related retroviral vectors.

A-71

THE STUDY OF GENE THERAPY USING PERIPHERAL BLOOD STEM CELLS (PBSC).

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Recent progress in gene therapy techniques lead to start the gene therapy to inherited metabolic diseases. In most protocols of gene therapy to inherited metabolic diseases, bone marrow stem cells are used as the targeting cells. However, PBSC, safely obtained from infants, is the candidate of the targeting cells for gene therapy in infants. In this study, to establish the protocol of gene therapy using PBSC, we examined the purification method of CD34+ cells from peripheral mononuclear cells obtained after G-CSF induction and the transduction efficiency of NeoR gene to CD34+ cells using retroviral vector. By the purification using immunomagnetic beads method after treatment of L-phenylalanine methylester, the purity of CD34+/CD33- cells was 74±18% and the recovery rate of CD34+ cells was 25±26%. Thus, this method is suitable to obtain CD34+ cells for gene therapy. The percents of CD34+ cells containig NeoR gene after retrovirus infection were 32 - 60% and 70 - 96% by G418 selection and PCR method, respectively. This result suggests that PBSC is the good targeting cells for gene therapy in infants.

A-72

IN VIVO CORRECTION OF OTC DEFICIENCY

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OTC deficiency is the most common and severe inborn error of the urea cycle in humans. Despite therapeutic advances, OTC deficiency remains without adequate treatment and mortality rates are high. For somatic gene therapy, adenovirus vectors provide an efficient system for gene delivery, but problems of toxicity and antigenicity remain. Efficient promoters which will reduce the amount of vectors required for treatment are needed. We constructed two recombinant adenovirus vectors, AdexCAGhOTC and AdexSRαhOTC, which harbor the human OTC gene, under control of CAG and SRα respectively. Both vectors were tested for expression in various cultured cell lines. OTC activity detected in the cells infected with AdexCAGhOTC was much higher than that seen with AdexSRαhOTC, in all cell lines. AdexCAGhOTC was also tested in spf^{ash} mice. Adult spf^{ash} mice were infected with 0.1 - 1.0 $\times 10^9$ PFU of AdexCAGhOTC and hepatic OTC activity was determined on the 7th day of post-injection. OTC activity detected in the liver ranged from 8% to 100% of control activity, in proportion to the dosage of vectors, whereas in the mock, it was 4±0.5%. Western blot analysis confirmed the hepatic OTC expression. Orotic acid in urine was dramatically reduced in all mice infected with AdexCAGhOTC. Morphological difference (gross or microscopic) between the liver infected AdexCAGhOTC and control was nil. Therefore, adenovirus vectors can correct OTC deficiency in vivo and efficient promoters are crucial for such vectors.

A-73

TRANSGENIC MICE EXPRESSING ANTISENSE GLUCOKINASE RNA.

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Mutations in the glucokinase gene have been found in patients with maturity-onset diabetes of the young (MODY), which has raised the possibility that a decrease in glucokinase activity may impair the glucose-stimulated insulin secretion in β cells, leading to diabetes. To test the effect of a reduced glucokinase activity in β cells on β cell function, we generated transgenic mice expressing an antisense glucokinase mRNA in β cells. The transgene construct contained the human insulin promoter linked to a 5' part of glucokinase cDNA of~280 bp long including the start codon and was designed to produce an RNA that hybridizes specifically to glucokinase mRNA molecules, resulting in reduced levels of glucokinase mRNA in cells expressing the transgene. Transgenic mice of line 15 showed an enhanced fasting glucose level. Islets isolated from this line of transgenic mice showed ~30% reduction in the glucose phosphorylating activity as compared with their normal littermates. This result supports the idea that glucose phosphorylation is a key step of the glucose sensing by β cells. IPGTT revealed that glucose tolerance was slightly impaired in this transgenic line only under the C57BL/6 genetic background, but not under the C3H background, suggesting polygenic nature of the blood glucose control.

A-74

EFFECT OF DRα EXPRESSION ON THE DEVELOPMENT AND PREVENTION OF DIABETES IN NOD MICE.

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Non-obese diabetic (NOD) mouse is an animal model for human type I diabetes; a T cell-mediated autoimmune disease in which T cells destroy insulin-secreting β cells in pancreatic islets of Langerhans. Previous studies have suggested that NOD mice expressing I-E as a result of the introduction of an I-Eα-chain (Eα) transgene are protected from the development of insulitis and hence of diabetes. To investigate the mechanism of this protection, we generated two strains of NOD mice expressing DRαEβ molecules and mice were monitored for the disease development. DRαEβ molecules have been shown to reconstitute the I-E controlled immune responses and T cell repertoire selection, however, DRα-NOD mice developed insulitis and diabetes to comparable levels to non-transgenic littermates. Furthermore, proliferative responses of T cells to GAD-derived peptides, which is shown to be a diabetogenic autoantigen, were observed in DRα-NOD, Eα-NOD and NOD mice. These results suggested difference in that structural substitutions between DRα and Eα molecules determine the expression and prevention of diabetes between DRα-NOD and Eα-NOD mice.

B-1

DENATURING GRADIENT GEL ELECTROPHORESIS OF PCR-AMPLIFIED DNA FRAGMENTS FOR DETECTION OF VARIATION IN DNA. PART 4. VARIATIONS OBSERVED IN p53 GENE. Norio TAKAHASHI, Hideo OMINE, Akiko MIURA, Junko KANEKO, Chiyoko SATOH (Dept. Genet., RERF, Hiroshima)

Denaturing gradient gel electrophoresis of PCR-amplified DNA (PCR-DGGE) is an effective method to detect subtle differences (*e.g.* single base substitutions or deletions and insertions of one to dozens of base pairs). We amplified four targets containing about 7.1 kb from the 3′ of intron 1 to the 5′ of exon 11 of p53 and examined them, using this technique. We also examined the overlapping 461 bp fragments in this area. Genomic DNA obtained from B-cell lines established on 100 families (fathers and mothers: 100 each, children: 124, total: 324 individuals) was used as samples. As a result, seven types of variants, in addition to the five types that are known to exist in normal populations, were observed in polymorphic proportions. Also, four types of rare variants were observed. All of these 16 types, excepting two types of the rare variants observed only in either of the parents, were ascertained to be hereditary variations. DNA sequencing revealed that 15 types of variations were single base substitutions and the remaining one type was insertion of 16 base pairs. All variants were observed in introns, excepting two that were identified as variants that cause amino acid substitution in the coding region. Many of the variations that exhibited polymorphisms were strongly linked with each other. They were classified into 10 haplotypes.

B-2

GENETIC VARIATION DETECTED BY QUANTITATIVE ANALYSIS OF END-LABELED GENOMIC DNA FRAGMENTS. Jun-ichi ASAKAWA[1], Rork KUICK[2], James V. NEEL[3], Samir M. HANASH[2], Satomichi KANEOKA[1], Mieko KODAIRA[1], Chiyoko SATOH[1] ([1]Dept. Genet., RERF, Hiroshima, [2]Dept. Pediatr., Univ. of Michigan, USA, [3]Dept. Hum. Genet., Univ. of Michigan, USA)

A refinement of the technique of two-dimensional electrophoresis of DNA fragments obtained from restriction-enzyme-digested genomic DNA permits the visualization on a single preparation of approximately 2000 DNA fragments varying in size from 1.0 to 4.5 kb in the first dimension and 0.3 to 2.0 kb in the second dimension. To enter into a genetic analysis, these fragments must exhibit positional and quantitative stability. With respect to the latter, if spots which are the product of two homologous DNA fragments are to be distinguished with the requisite accuracy from spots which are the product of only one fragment, the coefficient of variation of spot size should be < 0.12. At present, 482 of the spots in our preparations meet these standards. In an examination of preparations based on three Japanese mother/father/child trios, 43 of these 482 spots were found to exhibit variation which segregated within families according to Mendelian principles. We find that at present more than half of the variance in spot intensity results from steps in processing the image, with technical improvements a considerable number of additional spots should meet the criterion of $CV < 0.12$.

B-3

CLONING AND SEQUENCING OF GENETIC VARIANTS DETECTED BY TWO-DIMENSIONAL ELECTROPHORESIS OF END-LABELED GENOMIC DNA FRAGMENTS.

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We have been evaluating the efficiency of two-dimensional electrophoresis (2-DE) of end-labeled genomic restriction fragments in monitoring for mutations resulting in loss/gain/rearrangement events in DNA fragments. Restriction enzymes, *Not*I, *Eco*RV, and *Hinf*I are used to digest the DNA. Analysis of 2-DE DNA patterns of three Japanese mother-father-child trios revealed 482 spots each of which has a coefficient of variation (CV) for spot intensity less than 0.12; 43 of these spots were found to exhibit variation which segregated within families according to Mendelian principle. Among these 43 spots, we have cloned and characterized spot 589 and spot 1996, which seemed to be allelic. Sequence analysis of these fragments showed that approximately at position 700 bp from the *Not*I site, there is a *Hinf*I site sequence (..AGGAGTCGGG..) in the smaller DNA fragment of the spot 589, but the larger fragment of the spot 1996 does not have this site, being characterized by the sequence (..AGGAGTTGGG..). There are no other differences in the two sequences. Thus the allelic variation exhibited by these two fragments is shown to be due to a polymorphism in a *Hinf*I restriction site.

B-4

TETRANUCLEOTIDE REPEAT INSTABILITY IN THE CHILDREN OF ATOMIC BOMB SURVIVORS.

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In a pilot study to compare various types of DNA as potential targets for the detection of germinal mutations in the children of atomic bomb survivors, 50 exposed families including 64 children and 50 control families with 60 children from Hiroshima and Nagasaki have been examined for various DNA sequences. DNA samples were extracted from permanent cell lines established from B-lymphocytes.

Microsatellites are among the candidate targets. Mutation rate at the CCTT/CTTT-tetranucleotide repeat sequence in the colony stimulating factor-1 receptor (*CSF1R*) gene in Finland was reported to be 1.3% (range 0.3-2.7%) after examination of 234 meioses among 77 families. We examined the CCTT/CTTT-repeats in intron 2 and the TAGA-repeats in intron 6 of the *CSF1R* genes of the 100 families and detected two mutations each in the two repeat sequences. Thus, mutation rate was 0.8%/gamete at each of these repeat sequences. However, because the four mutations were detected in the children of the unexposed parents, no effects of parental exposure to radiation were observed for the tetranucleotide repeat instability, thus far.

B-5

CONSTRUCTION OF COSMID CONTIGS AND HIGH RESOLUTION RESTRICTION MAPS OF THE HUMAN IMMUNOGLOBULIN λ GENE LOCUS.

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To elucidate the complex structure of the human immunoglobulin λ gene locus, a chromosome 22 specific cosmid library was screened using four yeast artificial chromosomes and four variable region (V) gene segments as probes. By fingerprinting and chromosome walking, two contigs with a total length of 1.08 megabases were constructed using 200 cosmids. These contigs include all of the clones positive for the Vλ probes. High resolution physical maps were then generated, elucidating the locations of 67 unique *Eco* RI-*Hin* dIII segments positive to the Vλ probes, the VpreB gene, BCRL4 and a GGT related sequence. The two large contigs can be covered with only 35 cosmid. These minimum overlapping cosmids together with high resolution physical maps would serve as an excellent resource for large scale sequencing of this locus.

B-6

GENETIC POLYMORPHISM OF HLA-DQA1 AND DQB1 GENES.

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Full length cDNA sequences of 13 DQA1 and 13 DQB1 alleles were determined. Several sequencing errors in the published sequences (DQA1*0102, DQA1*03012, DQA1*0302, DQA1*05013, and DQB1*03031) were found and 3 new alleles (DQA1*01022, DQA1*0503, and DQB1*0202) were identified, which differed by exon 3 and/or exon 4 sequences from the known alleles (DQA1*01021, DQA1*05011 and DQB1*0201, respectively). The polymorphism in exon 3 and 4 of DQA1 and DQB1 genes were analyzed by PCR-SSOP method in Japanese and Norwegian populations and in B-lymphoblastoid cell lines homozygous for HLA region. DQA1 and DQB1 alleles of identical exon 2 sequences could be split into subtypes, DQA1*0101 and DQA1*0104, DQA1*01021 and DQA1*01022, DQA1*0301 and DQA1*0302, DQA1*05011, DQA1*05013 and DQA1*0503, DQB1*0201 and DQB1*0202. Each subtype represented strong linkage disequilibrium with specific DRB1 alleles. These observations suggested that DRB1-DQA1-DQB1 haplotypes had been evoluted separately.

B-7

HLA CLASS II ALLELES IN NATIVE JAPANESE (AINU) LIVING IN HIDAKA DISTRICT, HOKKAIDO. Makoto BANNAI, Katsushi TOKUNAGA*, Shinji HARIHARA**, Kiyoshi FUJISAWA, Takeo JUJI*, and Keiichi OMOTO*** (Tokyo Metropolitan Blood Center, *Japanese Red Cross Central Blood Center, **Dept. Anthropology, Tokyo Univ., ***International Research Center for Japanese Studies, Tokyo)

The Ainu people are considered to be relatively pure descendants of pre-agricultural native populations of northern Japan, while the major population of the present-day Japan (Wajin) are considered to be descendants mainly of postneolithic migrants. Polymorphisms of the HLA-DRB1, DRB3, and DQB1 alleles were investigated for 50 Ainu people living in Hidaka district, Hokkaido. High allele frequencies of DRB1*1401 and 1406 (20 and 17 %, respectively) were observed, and a novel allele DRB1*1106 was found with 5%. On the other hand, some of the common alleles in the Wajin (DRB1*1502, 1302, 0803 and 1501, 7-9% in allele frequency) were found with relatively low frequencies (1-2%) in the Ainu. DRB1*1106 was found thus far from only Singaporean Chinese and a Korean, and DRB1*1406 was a characteristic allele of some ethnics in Amerindians or North East Asians. These observations suggest the genetic links of the Ainu people with Amerindians and East Asians.

B-8

Comparison of bound peptides elluted from HLA-A2 subtypes molecules

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MHCclassI molecules bind 8~11mer peptide fragments derived from chiefly endogenious proteins,and this complex is recognized by TCR of CD8 Tcells. In autoimmune thyroid desease, Graves' desease and Hashimoto desease were both associated with HLA-A2,but by using our newly established DNA typing of HLA-A locus with the PCR-SSOP methpd revealed associated with different HLA-A2 subtype.To study the molecular basis of this association, we analysed the bound peptides elluted from HLA-A0201,A0206,and A0207 moiecules of EB transformed cell lines by protein sequencer analysis monitoring with mass spectrometry.
As repoted to date, in the HLA-A0201 prefered amino acids (aa) were L at position 2 (P2) and V,L, at P9. In the A0206 (only one residue was substituted at residue 9 to Y from F in the A0201) prefered aa were V,Q,L,I at P2 ,L,I,V at P3, and V,L at P9. And in the A0207 (only one residue was substituted at residue 99 to C from Y in the A0201) prefered aa were L at P2, D at P3, and L,V at P9. Our results exhibit that the naturally processed bound peptides are remarkablly different in the closely related HLA-A2 subtypes of A0201,A0206,and A0207,and will affect the immune response of each individuals.

B-9

POLYMORPHISM AT THE HLA-DRβ37 INDUCED CONFORMATIONAL CHANGES OF DR-BINDING PEPTIDES DISTINGUISHED BY A T CELL RECEPTOR. Yasuharu NISHIMURA, Yu-zhen CHEN, and Sho MATSUSHITA (Div. Immunogenet., Kumamoto Univ. Grad. Sch. Med. Sci., Kumamoto)

HLA-DR is a heterodimeric membrane protein consisted of monomorphic α and polymorphic β chains expressed on antigen presenting cells(APC). DR binds peptides derived from antigen processed by APC to present them to $CD4^+$ helper T cells. We have generated a T cell clone 5-32 specific to 15 amino acids(A.A.) long peptide M12p54-68 derived from a streptococcal M12 protein in the context of allogeneic DRB1*0403 as well as autologous DRB1*0406 which differs from DRB1*0403 only at A.A. residue 37, from Tyr to Ser substitution. By investigating binding ability to purified DR molecules and stimulatory activity to 5-32 of 154 analog peptides carrying single A.A. substitution at each A.A. residue of the M12p54-68, three residues 57, 60 and 62 were identified to be DR anchors, whereas the other three residues 58, 61 and 63 were recognition sites for the T cell receptor (TCR sites). Relatively restricted A.A. substitutions in TCR sites were allowed for stimulation of 5-32 as compared with DR anchors where substitutions to 5~8 A.A. still conserved T cell stimulatory activity. More than 50% of non-agonistic TCR site analog peptides exhibited antagonistic activity to TCR whereas DR anchor analogs did not. Up to 27 analog peptides were recognized by 5-32 very differently as agonists or antagonists between presentation by DRB1*0403 or 0406 molecules indicating that single A.A. polymorphism at DRβ37 induced conformational changes of DR-binding peptides whcih can be distinguished by the TCR.

B-10

IDENTIFICATION OF AN AUTOREACTIVE T-CELL EPITOPE BY COMPARATIVE STRUCTURAL ANALYSIS OF HLA-DR4-BINDING PEPTIDES. Sho MATSUSHITA[1], Shuji IKAGAWA[1], Keiji KOMORIYA[2], and Yasuharu NISHIMURA[1] ([1]Div. Immunogenet., Kumamoto Univ. Grad. Sch. Med. Sci., Kumamoto, [2]Teijin Inst. Biomed. Res., Hino)

A DR4-binding peptide AAFAALANAA identified in our previous study bound to DRB1*0405 as well as DRB1*0406 complex, which differ only at DRβ residues 37, 57, 74, and 86. Determination of the binding affinity of synthetic AAFAALANAA-based analogue peptides showed that substituting F to W or C; L to F, W or Y; and N to Q or S on AAFAALANAA changed the affinity substantially between DRB1*0405 and DRB1*0406. It is noteworthy that all patients with methimazole-induced insulin autoimmune syndrome are positive for DRB1*0406 and negative for DRB1*0405. Interestingly, the quantitative structural motif identified in this study successfully predicted that ^{8}TSICSLYQLE^{17} of human insulin α chain binds specifically to DRB1*0406 using its ^{10}IxxLxQ^{15} motif. Furthermore, a short-term T-cell line specific to human insulin established from a DRB1*0406-bearing individual did show reactivity with this peptide fragment. Thus, a reducing compound such as methimazole should cleave the disulfide bond on insulin (^{6}Cys-^{11}Cys)*in vivo* and allow DRB1*0406 complex to bind the linear fragment of insulin α chain, which may lead to the activation of self-insulin-specific T-helper cells.

B-11

SEQUENCE ANALYSIS OF HLA-B51 BOUND PEPTIDES DERIVED FROM HEALTHY DONORS AND PATIENTS WITH BECHET'S DISEASE.
Nobuhiro KAMIKAWAJI, Tohru SUDO, and Takehiko SASAZUKI (Dept. Genettics, Medical, Inst. Bioreg. Kyushu, Univ., Fukuoka)

Amino acid residue motif for peptides binding to HLA-B51 was identified by sequence analysis of reversed phase HPLC fractions containing endogenous peptides derived from HLA-B51 molecule. 15 different primary sequences were determined for HLA-B51 associated peptides by CID mass spectrum. 9 of which were eight aa in length. Common features among these peptides were Asp at position 1 and Ile or Val at COOH-terminus. HLA-B51 bound peptides among 5 B cell lines which were derived from three healthy donors and two patients with Bechet's disease, were compared using mass spectrometry. 27 major peptide ions were observed at the same HPLC fraction, the same retention time, and the same mass number in all 5 materials. The fact that the most endogenous peptides bound to HLA-B51 is almost the same between healthy donors and patients with Bechet's disease suggested that the processing, which include protease activities and transport mechanisms in the patients with Bechet's disease must not be impaired.

B-13

HLA-A NULL ALLELE WITH A STOP CODON GENERATED BY A POINT MUTATION.
Yoshihide ISHIKAWA, Katsushi TOKUNAGA, Hidenori TANAKA, Kouichi KASHIWASE, Tatsuya AKAZA, Kenji TADOKORO and Takeo JUJI (Dept. Research, Japanese Red Cross Central Blood Center, Tokyo)

A healthy individual having no serologically detectable HLA-A locus antigens was found. The individual TSU is a child of a consanguineous marriage. Results of a family study indicated that the individual is homozygous for A(blank)-B46-Cw1-DR8.1 haplotype. In our previous study, this haplotype frequently carried A*0207. Total RNAs extracted from peripheral blood were converted to cDNAs. The RT-PCR product, which has the same size as that of normally expressed gene, was easily obtained from the cDNAs with HLA-A specific primers. This result suggests that the mRNA of A-locus is normally produced in this individual. After cloning using a T-vector system, the nucleotide sequence from exon 1 to exon 8 was determined. The nucleotide sequence of this null allele was the same as that of A*0207 except for a single nucleotide which resulted in a stop codon in exon 4. Genomic DNAs from 29 unrelated panels, who expressed only one HLA-A antigen and HLA-B46, were analyzed by a PCR-SSO method. Although TSU's parents had the stop codon, none of the unrelated panels possessed this stop codon. Therefore, this null allele is likely to be a rare null allele generated by a single point mutation from A*0207.

B-14

MOLECULAR ANALYSIS OF C4 GENES ASSOCIATED WITH Rg:-1,-2 PHENOTYPES. Yoshihisa WATANABE, Hatsue TSUNEYAMA, Makoto UCHIKAWA, Katsushi TOKUNAGA, Kenji TADOKORO, and Takeo JUJI. (Japanese Red Cross Central Blood Center, Tokyo)

Rodgers (Rg) antigen is the determinant of the human C4 and usually located on the C4d fragment of C4A. In the previous study, nine Rg:-1,-2 donors were screened from 3088 donors serologically, and their C4 allotypes and the presence or absence of C4A gene were examined. In this study, the MHC class III gene organization of the Rg:-1,-2 donors were investigated. Genomic DNAs from four Rg:-1,-2 donors, who were identified as homozygous for C4AQ0 by C4 protein allotyping and seemed to have no C4A gene by specific amplification by PCR, were analyzed by pulsed-field gel electrophoresis. The results of the digestion with restriction endonuclease *Bss*H II and the hybridization with C4 or 21OH probe revealed that about 35kb deletion including C4A and 21OHA genes was detected. Conventional RFLP analysis using restriction endonuclease *Taq* I also detected only one C4B and one 21OHB fragments. These results indicate that they have only one set of C4/21OH genes on their genome. Other three Rg:-1,-2 donors had a C4A protein whose electromobility was abnormal. Two DNA samples of the donors were analyzed by PCR-RFLP and the results showed that their C4A genes may have nucleotide substitution(s) in the sequence coding for the Rg determinant.

B-15

ANALYSIS OF HLA-A GENE IN AUTOIMMUNE THYROID DISEASES.
Yukiji DATE, Akinori KIMURA, Tohru SUDO, and Takehiko SASAZUKI
(Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

A PCR-based DNA typing method for HLA-A gene was developed and used to investigate the association of HLA-A and autoimmune thyroid diseases. 87 patients with Graves disease and 100 patients with Hashimoto's thyroiditis were investigated. Highly polymorphic region of HLA-A gene was amplified by PCR and analyzed for the polymorphism by dot-blot hybridization with 84 types of SSOPs (sequence-specific oligonucleotide probes). This method enabled us to distinguish all HLA-A alleles at the DNA level. In the patients with Graves disease, frequency of A*0206 was significantly increased (14.5% vs 32.2% RR=2.79 p<0.0001 pc<0.005), while the frequencies of A*2402 and A*3302 were decreased (60.5% vs 48.3% RR=0.61 p<0.05, 18.5% vs 9.2% RR=0.47 p<0.05, respectively). In the case of Hashimoto's thyroiditis, frequency of A*0207 was significantly increased (8.1% vs 17.0% RR=2.35 p<0.01) and that of A*3302 was decreased (18.5% vs 6.0% RR=0.30 p<0.005). These findings suggest that HLA-A2 associated susceptibility to disease was mediated by different and specific subtype in Graves disease and Hashimoto's thyroiditis.

B-16

POSASIBLE CONTRIBUTION OF AMINO ACID SEQUENCE ON HLA-DR TO PATHOGENESIS OF PROGRESSIVE SYSTEMIC SCLEROSIS. Fujio TAKEUCHI, Keiichiro NAKANO, Akira YOSHIDA, Hirofumi YAMADA, Gi Hyun HONG, Hiromi NABETA, Makoto BANNAI*, Katsushi TOKUNAGA *, Koji ITO (Faculty of Medicine, University of Tokyo, Tokyo) and (*Department of Research, Japanese Red Cross, Tokyo)

Progressive systemic sclerosis (PSS) is an autoimmune disease with unknown etiology. The association of PSS to HLA has been reported but are controversial among ethnics. The contribution of HLA-DR to susceptibility of PSS was examined in 36 Japanese PSS and 104 normal subjects by polymerase chain reaction (PCR) method using specific primers and by PCR-SSCP (single-standard DNA conformation polymorphism) method. In PSS, a haplotype DRB1*1502-DRB5*0102 was significantly increased ($p<0.00004$), especially in anti-topoisomerase I antibody (a-Scl-70) positive patients ($p<0.00003$) and PSS with diffuse scleroderma ($p<0.00001$). DRB1*0802 was also increased in DRB1*1502 negative patients with a-Scl-70, ($p=0.033$) and in DRB1*1502 negative patients with diffuse scleroderma ($p=0.008$). Subsequently, 81.3% of a-Scl-70 positive patients and 93.8% of PSS patients with diffuse scleroderma showed either of these alleles. Furthermore the sharing of the particular amino acid sequence: ^{38}V and ^{67}FLED^{70}R, by DRB5*0102, DRB1*0802 and DR11 (associated with Caucasian PSS) would suggests a contribution of the sequence to the pathogenesis of PSS according to the shared epitope hypothesis.

B-17

Pseudoautosomal boundary (PAB1X and PAB1Y) - like sequence existing near boundary of long-range G+C% mosaic domains in the human MHC locus. Toshimichi IKEMURA, Tatsuo FUKAGAWA, Yasukazu NAKAMURA, Kimihiko SUGAYA, Ken-ichi MATSUMOTO (Dept. Evol. Genet., Natl. Inst. Genet. and Grad. Univ. for Advanced Studies, Mishima), Asako ANDO, and Hidetoshi INOKO (Dept. Molec. Life Science, Tokai Univ., Isehara)

The human genome is composed of long-range G+C% mosaic structures related to chromosomal bands. We had found the human MHC locus to be an example of Mb-level G+C% mosaic structures. Chromosome walking of 450 kb bridging MHC classes II and III and base-compositional analysis could precisely locate the boundary of the mosaic domains, disclosing a sharp G+C% transition. Near the transition point was a sequence highly homologous with the pseudoautosomal boundary of short arms of human sex chromosomes (PAB1X and PAB1Y) which is the interface between sex-specific and pseudoautosomal regions. Many PAB1XY-like sequences (PABLs) were detected by hybridization against genomic DNA, and the new sequences defined the complete form of PABLs of about 650 nt. Homologous sequences in the bovine genome were detected by hybridization, suggesting their evolutionarily stable maintenance and biological significance. Fukagawa et al., *Genomics*, in press.

B-18

DNA REPAIR FUNCTION OF THE *XPA* GENE. Kiyoji Tanaka, Yoshimichi Nakatsu, Masafumi Saijo, Isao Kuraoka, Toshiro Matsuda, Hironobu Nakane, Hiroaki Murai (Institute for Molecular and Cellular Biology, Osaka University, Osaka)

XPA is a zinc finger DNA-binding protein which is missing or altered in group A xeroderma pigmentosum (XP) cells and known to be involved in the damage-recognition step of the nucleotide excision repair (NER) processes. We identified the DNA binding domain of XPA. The region of XPA containing a C4 type zinc finger domain is sufficient for its preferential binding to damaged DNA. The E-cluster and the C-terminal regions which are conserved in XPA homologues are not necessary for the DNA binding activity of XPA. We speculated that they might be domains for protein-protein interactions which coordinate the NER processes. We therefore searched for proteins which binds to XPA using the yeast two hybrid system, and found four candidates which bind to XPA, two of which are unknown proteins. The interaction between two known proteins and XPA was confirmed by *in vitro* or *in vivo* experiments. We will disscuss the biological meaning of these interactions.

B-19

ALTERATIONS OF hMSH2 and hMLH1 GENES IN CANCER FAMILY SYNDROMES. Yasuhito YUASA, Yoshimitsu AKIYAMA, Hiromi NAGASAKI, Kazuo MARUYAMA (Dept. Hygiene & Oncology, Tokyo Medical and Dental University School of Medicine, Tokyo) and Tadashi NOMIZU (Dept. Surg., Hoshi General Hosp.)

Recentry, the causative genes for hereditary non-polyposis colorectal cancer(HNPCC), hMSH2 and hMLH1, have been cloned and idenified to be related to the DNA mismatch repair system. To clarify the carcinogenic prosess in HNPCC and other cancer family syndromes, we analyzed the hMSH2 gene in 12 HNPCC and 4 familial gastric cancer families using PCR-SSCP analyses. We detected mobility shifts in normal cells from 3 HNPCC and 1 familial gastric cancer patients. The mobility shifts were also seen in cancer cells from at least 1 case each from these two familial cancer syndoromes. We have not detected alterations in the hMLH1 gene so far. These results indicate that the hMSH2 gene may involve not only in HNPCC but also in the familial gastric cancer syndrome.

B-20

GERMLINE AND SOMATIC MUTATIONS OF HMSH2 GENE FOUND IN A COLON CANCER OF A PATIENT IN A CANCER PRONE FAMILY.
Akinori KIMURA, Haruhito HARADA, Seikoh YASUNAGA, Masanori FURUSE, Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka) and Ryuichi MIBU (1st. Dept. of Surgery, Kyushu Univ., Fukuoka)

To decipher the molecular basis of carcinogenesis in cancer prone families, non-polyposis colon cancers were examined for somatic mutations in microsatellite repeats (genomic instability) and in cancer-related genes (Ki-ras, p53 and APC). Genomic instability was preferentially found in the tumors from patients with hereditary non-polyposis colon cancer (HNPCC), and mutations in the Ki-ras gene were detected in 29% of the tumors with genomic instability. Screening of a mutation in the hMSH2 and hMLH1 genes showed that one out of 6 patients with HNPCC had germline (Cys 693 ter) and somatic (Tyr 757 ter) mutations of the hMSH2 gene in his colon cancer. RT-PCR analysis revealed that these mutations are in *cis* and, therefore, both hMSH2 alleles were defective in the tumor. These observations suggest that complete loss of functional hMSH2 gene causes the genomic instability and hense is involved in the carcinogenesis in cancer-prone families. However, other different target genes than p53 and APC genes may also be affected in the tumors with genomic instability, because somatic mutations were found less frequently in these tumors.

B-21

ABROGATION OF TUMORIGENICITY AND RESTORATION OF SERUM RESPONSE IN HUMAN COLON CANCER CELL LINES DISRUPTED AT ACTIVATED KI-RAS.
Masanori FURUSE, Mariko OHMORI, Shuji TOMITA, Daisuke MATSUZOE, Senji SHIRASAWA, and Takehiko SASAZUKI.(Dept. of Genetics, Medical Inst. of Bioreg., Kyushu Univ. Fukuoka)

To investigate the role of activated Ki-ras gene in carcinogenesis, we disrupted Ki-ras in two colon cancer cell lines, DLD-1 and HCT116, both of which have activated Ki-ras. Introducing a targeting vector containing neo-resistance gene and diphteria toxin fragment A gene, we obtained activated Ki-ras- disrupted progeny (HR-M) and normal Ki-ras-disrupted progeny(HR-N). HR-M showed reduced growth rate, soft agar cloning efficiency, and loss of tumorigenicity in nude mice. This finding indicates the critical role of activated Ki-ras gene in tumorigenicity in vivo and in vitro. We then examined serum responsiveness of immediate early genes in these cell lines. HR-N, which retains the activated Ki-ras, showed delayed serum response of c-myc, while the parental cell lines and HR-M showed the immediate serum response. On the other hand, HR-M showed reduced c-myc expression in exponentially growing state, and restoration of serum responsiveness of c-jun, c-fos genes. These findings indicate that normal Ki-ras would mediate serum response of c-myc gene and that the activated Ki-ras would enhance the expression of c-myc and inhibit the serum responsiveness of c-jun, c-fos genes.

B-22

IN VITRO GROWTH SUPPRESSION IN HUMAN ORAL SQUAMOUS CELL CARCINOMA CELL LINES BY THE INTRODUCTION OF CHROMOSOME #3. Narikazu UZAWA[1,2], Mitsuaki A. YOSHIDA[2], Mitsuo OSHIMURA[3], Tatsuro IKEUCHI[2] ([1]1st Dept. Oral Maxillofac. Surg., Fac. Dent. [2]Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ.,Tokyo, [3]Dept. Mol. Cell. Genet., Sch. Med., Tottori Univ.,Tottori)

Recent cytogenetic and molecular studies have revealed various genetic changes in oral squamous cell carcinomas (SCC). Loss of the short arm of chromosome 3 is one of the most frequent abnormalities in human head and neck cancers including oral SCC, suggesting the presence of one or more putative tumor suppressor gene(s) on #3p. To investigate the role of #3p loss in the development of oral SCC, we introduced a single normal chromosome 3 into human oral SCC cell lines, HSC-2, HSC-3, and HSC-4, all established in our laboratory, via microcell-mediated chromosome transfer. A total of 5 micro-cell hybrid clones with an intact donor chromosome were isolated and examined for their phenotypic properties. These clones exhibited a marked morphological change and a significant decrease in the growth rates _in vitro_. On the contrary, there were no phenotypic alterations in clones with a nomal chromosome 7 transferred as a control. Our findings provide additional evidence that a tumor suppressor gene(s) on #3p might be associated with the development of human oral SCC.

B-23

LOSS OF HETEROZYGOSITY AND SOMATIC MUTATION OF THE VON HIPPEL-LINDAU DISEASE GENE IN PRIMARY HUMAN RENAL CELL CARCINOMAS. Soichiro TORIGOE, Keiichi KONDO, Masahiro YAO, Naoki SAKAI, Yoshinobu KUBOTA, Masahiko HOSAKA, Taro SHUIN, (Dept. Radiol. [S. T.], Urol. [K. K., M. Y., N. S., Y. K., M. H., T. S.,], Yokohama City University School of Medicine, 3-9, Fukuura, Kanazawa-ku, Yokohama 236, Japan), and Micheal I. LERMAN, Berton ZBAR, (Lab. Immunobiol. National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702, U. S. A.)

We analysed 47 primary sporadic human renal cell carcinomas (39 clear cell and 8 non-clear cell) for mutations of the von Hippel-Lindau (VHL) tumor suppressor gene using the polymerase chain reaction and sigle strand conformational polymorphism analysis of DNA. All of the positive cases in single conformational polymorphism analyses were further characterized by direct sequencing. Somatic mutations were detected in 22 (56%) of 39 clear cell renal carcinomas including 15 deletions, 3 insertions, 3 missense mutations, and 1 nonsense mutation. Nineteen of these mutations predicted to produce truncation of the VHL protein. These mutations mainly occurred in the last one-third region of exons 1, 2, and 3. In addition, loss of heterozygosity of the VHL gene was observed in 16 (84%) of 19 informative clear cell carcinomas. These results show that the VHL tumor suppressopr gene is one of the major tumor suppresor genes in human renal cell carcinomas, especially in the clear cell type renal cell carcinoma. Clear cell carcinoma might be distinguished from other pathological types of renal cell carcinomas by molecular genetic techniques.

B-24

GENOMIC INSTABILITY IN CHILDHOOD SOLID TUMORS DETECTABLE BY SIMPLE REPEAT SEQUENCES. Hiroko YANAGISAWA[1], Keiko TADOKORO[1], Yasuhide HAYASHI[2], Masao YAMADA[1] ([1]Natl. Children's Med. Res. Ctr., Tokyo, [2]Dept. Pediatrics, Univ. Tokyo, Tokyo)

Genetic instability has become one of the most exiting topics in the field of human genetics due to new evidences recently found in cancers and genetic diseases. Patients with non-polyposis colon cancer have abnormal alleles detectable by PCR at microsatellite loci. This observation has led to reveal that such patients have a mutation in the gene functioning in a DNA repair process. Triplet repeat expansion has been shown as the molecular ethiology in several genetic diseases like Huntington's diseases and dentatorubral and pallidoluysian atrophy (DRPLA). Besides these distinctive microsatellites of the specific cases, only a few data have been available for DNA stability. We have analyzed microsatellite repeats including di-, tri- and tetra-nucleotide repeats in different sources, particularly in childhood solid tumors, like neuroblastoma, Wilms' tumor and germinal tumors, and compared to those in normal leukocytes of the same patient. Abnormal alleles were detected in tumor samples at a level from 0 to 3% depending on the loci, among which CAG repeats of the DRPLA gene was most unstable.

B-25

"WANDERING TRANSLOCATION (OUR THIRD CASE)" OF A TRISOMIC WHOLE q ARM OF CHROMOSOME #1 IN A BIPHENITYPIC LEUKEMIA. Tamiko SHINOHARA, Keiko KANNO (Dept. Human Cytogenetics, Japan Red Cross Med. Center, Tokyo), Yasuyuki INOUE, Kenji SUZUKI (Dept. Inter. Med., Japan Red Cross Med. Center, Tokyo) and Satoshi IWAI (BML Inc., Kawagoe City, Saitama Pref.)

The cytogenetic study of bone marrow cells from a 23 year old male with an acute biphenotypic leukemia is reported. He was pointed out leukocytosis associated with pain of his both knees, and was admitted to the Japan Red Cross Med. Centr. Hospital on Aug. 1993, At admission, his WBC was $14300/mm^3$ contained 53% blast cells. Bone marrow aspirate was hypercellular. Surface marker study showed that 43.0%, 95.7%, 57.8% and 95.1% of cells stained positively for CD13,CD19, CD33 and HLA-DR, respectively. He was diagnosed as mixed lineage leukemia. At diagnosis, chromosome study showed 45,X,-Y,t(4;16)(q21;q24),der(5)t(5;9)(q33;q13), -9.+m/46,XY. At relapse on Apr. 1994, the clonal evolution was observed with four types of additional abnormal chromosome: partial trisomy of the long arm of chromosome #1 associated with the telomeric segment of 1q or 14q or 15p and or mar. He was treated with combination chemotherapy including high dose Ara-C, but his disease resisted for chemotherapy. The unique feature of these translocation is discussed.

B-26

ASSOCIATION OF CHROMOSOME 12 REARRANGEMENTS IN UTERINE LEIOMYOMAS.

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We investigated chromosome 12 rearranements in uterine leiomyomas. A total of 56 uterine leiomyoma were cultured and harvested. Karyotypic analysis was performed on 46 tumors with GTG banding. The remaining ten cases yielded insufficient metaphase cells for conventional analysis. Eight cases showed cytogenetic rearrangements including one case with dir ins(4;12)(q33;q12qter). The ten unanalyzable cultures were studied with fluorescence in situ hybridization (FISH) using a chromosome 12 whole chromosome paint (WCP) probe, and a chromosome 12 alpha-satellite probe. One leiomyoma showed three signals with WCP only two of which were associated with alpha-satellite signals, a pattern most consistent with a structual rearrangement involving chromosome 12. Thus we were able to identify only two cases of chromosome 12 structual rearrangements among 56 uterine leiomyoma. In conclusion, Our data suggest that chromosome 12 rearrangements is not a major rearrangement in uterine leiomyomas.

B-27

TRISOMY OF CHROMOSOME 7 ANALYZED BY FLUORESCENCE IN SITU HYBRIDIZATION IN COLONIC POLYPS FROM THE PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS

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Trisomy 7 is frequently observed in cultured mitotic cells of colonic polyps from the patients with familial adenomatous polyposis (FAP). In order to evaluate the role(s) of trisomy 7 in the development and/or progression of colonic polyps, we analyzed the frequency of trisomy 7 in interphase cells by fluorescent in situ hybridization (FISH) and compared the results with those obtained by chromosome analyses. A total of 41 specimens from 14 patients were analyzed by FISH with α-satellite DNA probe (D7Z1). The frequency of trisomy 7 in 25 specimens, in which trisomy 7 was observed by chromosome analyses, varied from 4～72%. The remaining 16 specimens in which trisomy 7 was not chromosomally identified also showed trisomy 7 with frequencies of 3～86%. The trisomy 7 frequencies were not correlated with both the size and histological type of polyps. Of particular interest in this study was the finding that all of the polyps studied here by interphase FISH demonstrated a gain of chromosome 7. Our findings suggest that trisomy 7 may be one of the genetic changes associated with the development and/or progression of colonic polyps in the FAP patients.

B-28

A CYTOGENETIC INVESTIGATION OF 11;19 TRANSLOCATED LEUKEMIAS USING MICRO-FISH.

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We have reported at this Annual Meeting in 1990 that there were three types of reciprocal translocation in 11;19 translocated leukemias which involved the long arm of chromosome 11 and the short arm of chromosome 19. Especially, translocation t(11;19)(q23;p13.1) might have been misinterpreted as normal karyotype so far because of its small changes of arm length accompanied with the translocation. Clinically, it was suggested that this type of leukemias was clearly differentiated from t(11;19)(q23;p13.3) type. Translocation t(11;19)(q23;p13.1) was only observed in myeloid lineage leukemias, while translocation t(11;19)(q23;p13.3) was observed in acute lymphoblastic or biphenotypic leukemias. Recently, this observation was supported by Huret et al. [Leukemia 7:152-160(1993)].
We performed a cytogenetic investigation using Micro-FISH for the above two types of 11;19 translocated leukemias. Regional specific painting probes were constructed with microdissected fragments from 19p13.2 and 11q23-ter. In the result, the dual-color FISH using 19p13.2 and 11q23-ter regional specific probes confirmed the difference of breakpoints between p13.1 and 13.3 in 11;19 translocated leukemias.

B-29

DETECTION OF THE IgH/BCL2 FUSION GENE IN B-NHL CARRYING THE TRANSLOCATION t(14;18)(q32;q21) BY FLUORESCENCE IN SITU HYBRIDIZATION USING ALU-PCR AMPLIFIED YEAST ARTIFICIAL CHROMOSOME (YAC) CLONES CONTAINING THE HUMAN IgH AND BCL2 GENE LOCUS. Yutaka UEDA, Masafumi TANIWAKI, Kazuhiro NISHIDA, Shinichi MISAWA, Kei KASHIMA(3rd. Dept. Med.,Kyoto Pref. Univ. Med., Kyoto), Teruyuki TAKASHIMA(Dept. Med., Rakuwakai Marutamachi Hospital, Kyoto), Norimitsu KADOWAKI(1st. Dept. Med., Kyoto Univ., Kyoto) and Shiro FUKUHARA(1st. Dept. Med., Kansai Medical Univ., Ohsaka)

The IgH/BCL2 fusion gene was detected in metaphase spreads and interphase nuclei carrying the translocation t(14;18)(q32.3;q21.3) by double-color fluorescence in situ hybridization (FISH). Using yeast artificial chromosome (YAC) clones containing the human IgH gene and BCL2 gene, fusion genes were successfully visualized as yellow signal or doublet of red-green spots. In interphase nuclei, the percentage of fusion signals ranged from 90% to 95% in three cell lines and from 24.5% to 50% in four patients with B-NHL. The percentage of false possitive nuclei was only 0.6%. Our results indicate that the current aproach is fast and sensitive method for the detection of translocation t(14;18)(q32.3;q21.3) specific for B-cell malignancies.

B-30

INTERPHASE DETECTION OF BREAKPOINTS OF 14Q32 TRANSLOCATIONS IN B-CELL MALIGNANCIES BY DOUBLE-COLOR FLUORESCENCE *IN SITU* HYBRIDIZATION. Masafumi TANIWAKI, Yutaka UEDA, Teruyuki TAKASHIMA, Kazuhiro NISHIDA, Shigeo HORIIKE, Shinichi MISAWA, Kei KASHIMA, Tatsuo ABE (Depts. Med. and Hygiene, Kyoto Pref. Univ. Med. Kyoto), and Shiro FUKUHARA (1st Dept. Med. Kansai Medical School, Osaka)

Breakpoints of 14q32 translocations found in B-cell malignanciies were delineated specifically in both metaphase spreads and interphase nuclei by double-color fluorescence *in situ* hybridization (FISH) using bacteriophage clones containing the human immunoglobulin gamma chain gene locus (Igγ) and a cosmid clone, CY24-68, containing V_H segments. Incidence of interphase nuclei with a signal of Igγ clearly separated from that of CY24-68 ranges from 45 to 72% in three patients. In two cell lines carrying t(8;14), the signal of Igγ was detected at the breakpoint region 8q24.1 in addition to the der(14), indicating that translocation event occurred within the Igγ locus. The current approach provides a rapid diagnostic aid for the detection of 14q32 translocations in B-cell malignancies in routine cytogenetic studies.

B-31

THE WILSON DISEASE *WD* GENE IS HOMOLOGOUS TO THE LEC RAT *hts* GENE. **Takao ONO, Risaku HUKUMOTO, Shigeyuki TAKADA, and M.C. YOSHIDA (Chromosome Res. Unit, Fac. of Sci., and Cytogenet. Lab., Grad. School of Environmental Earth Sci., Hokkaido Univ., Sapporo)**

The LEC rat is a mutant strain which develops hereditary hepatitis and subsequently liver cancer. The LEC rat shares many clinical and biochemical features with Wilson disease, such as a low level of ceruloplasmin in plasma and copper accumulation in the liver. These observations suggest that hepatitis and liver cancer are caused by copper accumulation and that the LEC is a model for Wilson disease. An autosomal recessive gene is responsible for each disease, namely *hts* for LEC rats and *WD* for Wilson disease.

To test whether the LEC rat is a model for Wilson disease, we analyzed the linkage between hepatitis and *WD* gene using 71 backcross [(WKAH x LEC)x LEC] rats and human WD cDNA probes. Our results showed that identical segregation and no recombination event between *hts* and *WD* genes, indicating that the *hts* is homologous *WD* gene. Moreover, partial deletion of *WD* gene was found in LEC rats, similar to that of Wilson disease. The rat *WD* gene is assigned to 16q12.2-q12.4, and no synteny with *RB1* and *ESD* as shown in human was found.

B-32

FAMILY HISTORY AND BREAST CANCER RISK IN JAPAN.

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To provide a comprehensive assessment of family history(FH) and breast cancer(BC) risk, we have studied 132 families with family history of BC. Pedigree with family history (Family aggregation :FA) were defined as a proband's family with a first-degree or a second-degree relative with BC. To compare with FA, we selected 132 control families of sporadic cases by adjusted of operation age of the proband. Results 1) Familial Risk. The OR(odds ratio) for the women with her FH of BC was 1.48 (95% CI, 1.14 - 1.93). The OR for women with affected mother of FA-cases was 1.33 and the OR for women with unaffected mother of FA-cases was 1.52. A slightly greater risk was not observed if a woman's mother was affected. 2)The proband has expected to have one affected sisters of BC out of four sisters. 3) If the proband has a affected sister with BC, A slightly increased risk of any type of cancer was observed (OR, 1.79 CI, 0.86 to 3.71).

B-33

FAMILY HISTORY OF CHILDHOOD LEUKEMIA AND RELATED CONDITIONS

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Family history of childhood leukemia and related conditions were studied based on 29,804 cases in the Japan Children's Cancer Registry diagnosed between 1969 and 1992. The rate of probands whose sib had leukemia was significantly higher among probands with leukemia (0.225%) than among probands with other types of childhood cancer (0.045%). The type of leukemia of the sibs was the same as the proband's in 77.6% of the cases. The rate of the probands whose sib had a malignant lymphoma was significantly higher among malignant lymphoma probands (0.284%) than among probands with other childhood cancers (0.007%). Familial erythrophagocytic lympho histiocytosis (FEL), known as a recessive disease, affected only sibs and showed a high rate of parental consanguinity (37.5%). Hodgkin's disease also affected only sibs, and the parental consanguinity was high. On the other hand, leukemia and non-Hodgkin's lymphoma showed a dominant inheritance pattern. Parental consanguinity was not high. Leukemia affected more frequently cousins, aunts/uncles and parents in leukemia probands than in probands with other cancers. Non-Hodgkin's lymphoma more frequently affected cousins and/or parents of probands with a malignant lymphoma than the other probands.

B-34

GERM CELL TUMORS AND DOWN SYNDROME

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Some 232 Down syndrome (DS) cases (0.78%) associated with particular types of children with cancer among 29,804 cancer children were entered in the Japan Children's Cancer Registry in 1969-1992. This reflected a significant increase in Down syndrome as compared to the incidence (1.101%) reported in the Birth Defects Monitoring, Tokyo in the years 1980-1988. Of various types of childhood cancer in the Registry, leukemia, brain tumors, retroperitoneal tumors (malignant and benign) and genital tumors were significantly increased in DS. Major types of leukemia associated with DS were acute myelogenous leukemia, particularly M7 (FAB classification), which developed in neonatal or infancy periods, and acute lymphatic leukemia with common surface antigen, which developed in infancy to school age children with DS in the cases after 1985, when detailed classification of leukemia was introduced in the Registry. Twenty solid tumors were associated with DS including 2 non-glioma-type teratomas out of 4 brain tumors, 8 retroperitoneal teratomas (malignant and benign), 4 fetal cancers of testis, 1 germ cell tumor of ovary and 1 coccygeal epidermal cyst, indicating a high risk for germ cell tumors in DS as well as for leukemia.

B-35

APPLICATION OF DNA POLYMORPHISMS TO PATERNITY TESTING AND ALLELE FREQUENCIES.

Tamiko NAKAJIMA, Takasumi MATSUKI and Ken FURUKAWA (Dept. Legal Medicine, Gunma Univ. Sch. Med., Maebashi)

DNA typing provides a powerful tool for paternity testing and individual identification. We have analyzed three DNA polymorphisms (MZ1.3, MCT118, HLA-DQα). Typing with multilocus minisatellite probe MZ1.3 was performed by southern hybridization procedure. Typing of a single locus VNTR (MCT118) and HLA-DQα were detected by using PCR amplification followed by polyacrylamide gel electrophoresis and by hybridization with sequence-specific probes by reverse dot blotting, respectively. In DNA fingerprinting patterns with MZ1.3, on the average 18.3 polymorphic fragments were observed over 4.3 kb ranges after digested with Hinf I enzyme. We have calculated band sharing rates of MZ1.3 from nine paternity testing cases. The mean values of the rates were 55.7% for related and 16.7% for unrelated individuals. Allele and genotype frequencies of MCT118 were determined in unrelated 129 DNA samples. Twenty-two alleles were observed between 16 and 42 repeating numbers. The number's 24, 18, 30 and 28 were common alleles. There were 55 genotypes observed and 24/30, 18/24, 18/28 and 24/28 were common in our study. We have also tested HLA-DQα allele frequencies of 54 DNA samples. DQ1.3 and DQ3 alleles had high frequencies and DQ2 had the lowest one in 6 alleles. The commonest genotype was DQ3/3 in 13 genotypes. These three genetic markers seemed to be reliable on paternity testing and individual identification in our study.

B-36

STUDY ON PCR-RFLP ANALYSIS TO A2M(2) ALLOTYPING

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Hideo MATSUMOTO, Yasuhiko MIZOI (Dept. Legal Med., Osaka Med. Coll., Takatsuki)

We studied A2m allotyping system using PCR-RFLP analysis. Because the amino acid substitutions associated with the allotypic determinants for A2m are located at position 411, 428, 458 and 467, we designed two mismatch primers to detect TAC(Tyr) and GAG(Glu) at positions 411 and 428, respectively. A2m(1) and A2m(2) gene frequencies for 75 Japanese samples were calculated at 0.462 and 0.538, respectively. All the samples carrying axg haplotype were found to be defined as A2m(1) homozygote (χ^2=4.3495、P<0.05), the finding which confirmed the previous data (Matsumoto, 1973). Although two synonymous GCC and GCT codons at position 421 were reported to exist in *A2m*1* and *A2m*2*, respectively, we found that the GCC codon also occurred in *A2m*2* and never did in the alleles associated with Gm afb1b3.

B-37

SEQUENCE POLYMORPHISMS IN EXONS OF THE COAGULATION FACTOR XIII a SUBUNIT GENE.

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Sequence polymorphisms were detected in 7 sites of exons 2, 5, 8, 12, and 14 of the coagulation factor XIII a subunit gene (F13A) for Finn, Russian, and German populations by using PCR-SSCP and sequencing. A/C and A/G substitutions at codon 331 in exon 8 and codon 567 in exon 12, respectively, result in no amino acid changes, and G/T and A/T exchanges at codon 34 in exon 2 and at codon 204 in exon 5, respectively, lead to amino acid changes but to no surface charges of their products. We reported recently that sequence polymorphisms at codon 564 in exon 12 and codons 650-651 in exon 14 determine the four alleles (*F13A*1A, F13A*1B, F13A*2A, F13A*2B*) defined by IEF (Hum Genet 94:129-135). An additional variation in the sequences of codons 650-651 of the *F13A*2A* and *F13A*2B* alleles was disclosed, occurring as a GTT・CAG sequence as predicted in the previous paper. Determination of the nucleotide changes in the 5 exons for 108 samples revealed that at least 18 alleles with different sequences occur in the populations. These alleles were generated through point mutations and more frequently through intragenic crossing-over events.

B-38

DNA POLYMORPHISM OF ABO BLOOD GROUP GENE

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We investigated polymorphism of ABO blood group gene in donors by PCR-SSCP method and found 13 different alleles. The number of alleles in each subgroup was 4 (tentatively called ABO*A01, A02, A03 and A04) for A^1, 3 (ABO*B01, B02 and B03) for B, and 6 (ABO*O01, O02, O03, O04, O05 and O06) for O. The nucleotide sequence analysis revealed specific mutations of the alleles. In Japanese, A02 and B01 were the predominant alleles among the each subgroup at a frequency of 82% and 97%, respectively, whereas at the subgroup O, two major alleles, O01 (44%) and O04 (52%), were observed. Based on these observations, we have extended our study to other subgroups, such as A^2, A^x, and A^{el}. Consequently, we found additional 14 alleles in these subgroups. These results will be useful for the establishment of ABO genotyping as well as the elucidation of epitopes of serological specificities.

B-39

O-GENE DETECTION IN THE ABO BLOOD GROUP SYSTEM AND IT'S APPLICATION TO PATERNITY TESTING.

Takasumi MATSUKI, Tamiko NAKAJIMA AND Ken FURUKAWA (Department of Legal Medicine, Gunma University School of Medicine, Maebashi)

We have reported a fast and easy method for the detection of O-gene in the ABO blood group system (Jpn J Human Genet 39:293-297, 1994). Our method included allele-specific amplification using PCR followed by polyacrylamide gel electrophoresis and silver staining. We analyzed 148 unrelated DNA samples. O-gene product was detected in 54, 39 and 32 DNA samples from blood group O, A and B persons, respectively. No O-gene product was appeared in 7, 5 and 11 samples from A, B and AB persons, respectively. Genotype frequencies from these observed data fitted in with calculated genotype frequencies from phenotype data determined by serologic analyses.

We also analyzed 17 families with 4 or 5 members and 8 paternity testing cases. ABO genotypes of these cases were compatible with results from serologic blood groups or serum types, and DNA polymorphism like MCT118, MZ-1.3 and HLA-DQα. No case was found in which paternity was excluded by the ABO genotypes in this study.

B-40

The polymorphism of human O^6-methylguanine-DNA methyl-transferase. Masako ABE(Dept. Clin. Genet., Med. Inst. of Bioregulation, Kyushu Univ., Beppu), Makoto OTSUKA (Dept. Radiol.), Mutsuo SEKIGUCHI(Dept. Biochem., Fukuoka), Tomokazu SUZUKI(Dept. Clin. Genet.)

O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repairing enzyme that removes methyl group from O^6-methyl-guanine-DNA. Therefore it is expected that MGMT plays a role at the initiating stage in chemical carcinogenesis by alkylating agents. This eznyme activities have inter-individual differences. We investigated whether the polymorphism might exit in the MGMT coding region in normal subjects and patients with colorectal cancer. Using DNA extracted from leukocytes, PCR was performed for 4 coding exons, followed by SSCP analysis. We identified two variants (V1, V2). Direct sequencing of exon3 revealed that in V1 a C→T transition at nt. 290 causing a single amino acid change (Leu^{84}→ Phe) was combined with an additional silent C→T transition at nt.199. V2 had G→C point mutation at nt. 235, changing Try^{65} to Cys. The allele frequencies of V1 were 0.156 in normal subjects and 0.182 in patients. V2 was detected only in an individual in each group. The distribution of these variants was not significantly different between normal and patient groups.

B-41

MOLECULAR ANALYSIS OF VITAMIN D-BINDING PROTEIN (GC) VARIANT ALLELES.
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Molecular basis for seven GC variant alleles specific for the Asian populations was studied. A synonymous T to C transition was observed at the third nucleotide of codon 283 and common GC*1F was divided into two alleles (GC*1F^C and GC*1F^T). Table 1 summarizes the mutations found in the seven GC variant alleles. All the mutations resulted from A/G and C/T transitions. Thus, GC*1A2 has evolved through three mutational events: GC*1F^T→ GC*1F^C→ GC*1A3→ GC*1A2. These findings also provide interesting information on forensic and anthropological fields.

Table 1. Mutations in 7 GC variants.

Variant alleles	Base alleles	Mutations			
GC*1A2	GC*1A3	Ser^{335}	AGC→	Gly	GGC
GC*1A3	GC*1F^C	Arg^{429}	CGC→	Cys	TGC
GC*1A8	GC*1S	Arg^5	CGG→	Trp	TGG
GC*1A9	GC*1F^T	Arg^5	CGG→	Gln	CAG
GC*1C2	GC*1S	Leu^{238}	CTA→	Pro	CCA
GC*1C35	GC*1S	Lys^{377}	AAG→	Arg	AGG
GC*2A7	GC*2	Asn^{244}	AAC→	Asp	GAC

B-42

STRUCTURE OF THE HUMAN DEOXYRIBONUCLEASE I GENE AND IDENTIFICATION OF ITS POLYMORPHIC SITE.

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(Dept. of Legal Med., Fukui Medical School, Fukui)

The objectives of this study were to elucidate the structural organization of the gene for human deoxyribonuclease I (DNase I). In order to construct the organization of this gene, we utilized a combination of direct PCR-amplification of human genomic DNA and isolation of the overlapping clones from a cosmid human genomic library. DNase I gene was approximately 3.2 kb long and comprised 9 (I-IX) exons, and its complete nucleotide sequences were dteremined. The first exon contained only the non-coding sequences of mRNA. In addition to several putative regulatory elements, TATA-like and CAAT-like sequences were found in the region upstream of the initiation codon. The isoelectric focusing patterns of human DNase I exhibited protein polymorphism. A comparison of the entire translated sequences of the DNase I gene from individuals with DNase I phenotypes 1 and 2 revealed only one nucleotide residue difference in exon VIII, A for phenotype 1 and G for phenotype 2, thus producing Gln and Arg amino acid substitutions respectively at position 222 from the N-terminus of the mature enzyme.

Ref.) Yasuda et al. (1995) Ann. Hum. Genet., 59, 1-15.

B-43

GENETIC POLYMORPHISM OF DEOXYRIBONUCLEASE I DETECTED IN HUMAN SALIVA

Haruo TAKESHITA, Etsuko TENJO, Toshihiro YASUDA, Daita NADANO, Reiko IIDA, Koichiro KISHI (Dept. Legal Med., Fukui Med. Sch., Fukui) and Masao NAKANAGA (Dept. 2nd Int. Med., Fukui Med. Sch., Fukui)

The activity of salivary deoxyribonuclease I (DNase I) was determined to be 0.56 $\pm$ 0.565 units per mL, indicating that human saliva contained relatively high DNase I activity. Isoelectric focusing of whole saliva samples on polyacrylamide gels (pH 3.5-5), followed by dried agarose film overlay detection, was employed to determine the type of salivary DNase I. Since this detection method had not only a high sensitivity, but also a high band resolution, it was possible to determine DNase I types from saliva samples of 2-5 μL. Pretreatment of saliva samples with neuraminidase simplified the isozyme pattern and enhanced the sensitivity. The DNase I types in all 199 saliva samples showed a good correlation with the types found in the corresponding blood, semen, and urine samples.

Ref.) Tenjo et al. Electrophoresis, 14, 1042, 1993.

B-44

ABO Genotyping of Fingerprints by the PCR-RFLP Method. Masahiro SASAKI, Tohru FUKUSHIMA, Hiroshi SHIONO (Department of Legal Medicine, Asahikawa Medical College)

DNA typing of the ABO blood groups from single fingerprints was examined. Genomic DNAs were extracted from fingerprints on slide glasses by a simple method with NaI and N-lauroyl-sarcocinate, and then subjected to the PCR (Polymerase chain reaction). Two PCR fragments spanning positions 258 and 700 of the cDNA sequence of the ABO locus were amplified. Then, the PCR products were digested with a restriction enzyme, Kpn-1 or Msp-1, for each site. The digested PCR products were analyzed by electrophoresis on polyacrylamide gels for RFLP (restriction-fragment length polymorphism). Bands on the gel were visualized by silver staining. Genotyping from single fingerprints after treatment with aluminium powder was also possible by these techniques.

B-45

DNA ANALYSIS OF THE THREE LORDS OF THE DATE CLAN BY HLA CLASS II GENOTYPING. Rieko UCHIHI, Toshimichi YAMAMOTO, Hideki NOZAWA, Keiji TAMAKI, Yoshinao KATSUMATA (Dept. Legal Med., Nagoya Univ. Sch. Med., Nagoya), Tomowo OZAWA (Dept. Earth Science, Faculty of Science, Nagoya Univ., Nagoya) and Tadasu K. YAMADA (Dept. Zoology, National Science Museum, Tokyo)

The Human leukocyte antigen (HLA) class II genotypes of the three generations in the Japanese feudal lords of the Date Clan, Lord Masamune Date (1567-1636), Lord Tadamune Date (1599-1658) and Lord Tsunamune Date (1640-1711) were determined. The hair samples of Masamune and Tsunamune and the soft tissue of Tadamune were excavated with the skeletons from each mausoleum, and their genomic DNAs were purified using the cationic detergent cetyl-trimethyl ammonium bromide (CTAB). Although only small amounts of DNA had been recovered from those specimens, they could successfully be amplified by a semi-nested PCR for HLA-DQA1 gene and by reamplification for HLA-DPB1 gene. The PCR products were typed by the dot blot hybridization with the sequence-specific oligonucleotide (SSO) probes. It appeared that the three lords share the same alleles, DQA1*0301 and DPB1*0402. Therefore, the result showed no exclusion of their paternities. Since the genomic DNA recovered from the tissue remain or hairs as old as 300-350 years could be typed, the present method seems to be available for the DNA analysis of ancient materials.

B-46

EVALUATION OF POPULATION-SPECIFIC ALLELES BY MVR-PCR ANALYSIS. Keiji TAMAKI, Xiu Lin HUANG, Toshimichi YAMAMOTO, Rieko UCHIHI, Hideki NOZAWA, Yoshinao KATSUMATA (Dept. Legal Med., Nagoya Univ. Sch. Med., Nagoya) Alec J. JEFFREYS (Dept. Genet., Univ. Leicester, Leicester)

Minisatellite variant repeat mapping by the polymerase chain reaction (MVR-PCR) is a new approach to assessing variation within minisatellites. MS32 alleles have previously been shown to contain two major classes of 29bp repeat units, designated **a**-type and **t**-type, which differ by a single G-A transition. These two repeat types show highly diverse interspersion patterns within different alleles.However, different alleles show significant similarities in repeat arrays.All pairwise comparisons of the MVR haplotypes of 302 different Japanses alleles were performed by the dot matrix method. The heuristic alignment approach showed that 75.5% of the alleles could be classified into 31 different groups. The majority of inter-allelic differences in repeat copy number and variant repeat interspersion pattern cluster at the extreme beginning of the tandem repeat array, involving complex inter-allelic gene conversion events. All Japanese alleles were also compared with 754 alleles in other ethnic populations (Caucasian, African and Asian). Groups of Japanese alleles were largely population-specific, and only a few Asian alleles could be classified into some Japanese groups. MVR analysis is so informative of allelic internal variation that it seems to be a powerful tool for the investigation of human divergence.

B-47

MOLECULAR ANALYSIS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS IN THE SOLOMON ISLANDS. Akira HIRONO, Shiro MIWA (Okinaka Memorial Institute for Medical Research, Tokyo), Hisaichi FUJII (Dept. Transfusion Medicine, Tokyo Women's Medical College, Tokyo) and Akira ISHII (Dept. Parasitology, National Institute of Health, Tokyo)

Using non-radioisotopic polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis, we identified two types of mutants in 27 glucose-6-phosphate dehydrogenase (G6PD) deficient subjects from villages near Honiara, the capital of the Solomon Islands, on Guadalcanal Island. One mutant bore a single base substitution of C to T at nucleotide 1360 predicting an Arg to Cys substitution at residue 454 that was already reported to cause a class II variant G6PD Maewo. The other mutant carried two missense mutations: an A to G substitution at nucleotide 99 predicting an Ile to Met change at amino acid 33 in addition to the common 1360 C→ T mutation. The 99 A→G was unique and caused a new variant G6PD Honiara in combination with the 1360 C→T. The finding of the same mutation in G6PD Honiara as was found in G6PD Maewo strongly suggests that the 99 A→G occurred in an individual with G6PD Maewo.

B-48

VNTR POLYMORPHISM AT D1S80 LOCUS IN THE JAPANESE POPULATION,

Satoko ITO, Kouji NARAHARA, Shinsuke NINOMIYA, Kazushiro TSUJI, Yuji YOKOYAMA, Yoshiki SEINO (Dept. Pediatr., Okayama Univeristy Medical School)

Misdiagnosis due to naming error or maternal cell contamination of samples is challenging in prenatal diagnosis. We determined types and frequencity of VNTR polymorphism at the D1S80 locus in 82 unrelated Japanese , using PCR amplification and agarose electrophoresis. There were at least 19 allels, ranging in length from 380 to 740 bp. These alleles were rather evenly distributed, and the heterozygosity rate was 81 %. This method was successfully applied to discriminating the fetal genotype from maternal one in 10 amniotic fluid or chorionic villus samples. PCR amplification of VNTR polymorphism at the D1S80 locus is simple and useful in identifying preventable misdiagnosis in prenatal diagnosis.

B-49

A Y-ASSOCIATED ALLELE IS SHARED AMONG A FEW ETHNIC GROUPS OF EAST ASIA

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(Dept. Hum. Genet. Univ. Tokyo, Dept. Pediat., Nat. Cheng Kung Univ., Taiwan, 6 other Dept.)

In our previous study(Nakagome et al. 1992), both of Y-associated alleles, Y1 and Y2, were detected in Japanese and Koreans, but only the Y1 allele was detected in each of other populations including Chinese in both Beijin and Guangzhou areas, Caucasians, Africans, and Jewish. In the present study, these observations were extended to other ethnic groups in East Asia. Evenks in central Siberia and Khalkhs in Mongolia had only the Y1 allele. Fo-lo and Hakka, in Taiwan had both of the Y1 and the Y2 alleles. Three of the eight Y2-positive men, 2 Fo-lo and a Hakka, shared family name Chen. Both Hakka people and ancesters of Chen families could be traced to the Province of Henan in northern China in early 4th century. They arrived in Fujian/Guangdong area in the south-east China *via* various routes and then some of them migrated to Taiwan in the 18th century. It is tempting to speculate that the Y2 allele may be originated from an ancestral population in Henan from which, Japanese, Koreans, and some of the Taiwanese diverged.

B-50

THE RECENT AFRICAN ORIGIN OF MODERN HUMANS REVEALED BY COMPLETE SEQUENCES OF HOMINOID MITOCHONDRIAL DNA.
Satoshi HORAI, Kenji HAYASAKA, Rumi KONDO, Kazuo TSUGANE (Dept. Human Genet., National Institute of Genetics, Mishima) and Naoyuki TAKAHATA (The Graduate Univ. for Advanced Studies, Kanagawa)

We analyzed the complete mitochondrial (mt) DNA sequences of three humans (African, European and Japanese), three African apes (common and pygmy chimpanzees, and gorilla), and one orangutan in an attempt to estimate most accurately the substitution rates and divergence times of hominoid mtDNAs. Nonsynonymous substitutions and substitutions in RNA genes have accumulated with an approximately clock-like regularity. From these substitutions and under the assumption that the orangutan and African apes diverged 13 million years ago, we obtained a divergence time for humans and chimpanzees of 4.9 million years. This divergence time permitted calibration of the synonymous substitution rate (3.89×10^{-8}/site/year). To obtain the substitution rate in the D (displacement)-loop region, we compared the three human mtDNAs and measured the relative abundance of substitutions in the D-loop region and at synonymous sites. The estimated substitution rate in the D-loop region was 7.00×10^{-8}/site/year. Using both synonymous and D-loop substitutions, we inferred the age of the last common ancestor of the human mtDNAs as 143,000 ± 18,000 years. The shallow ancestry of human mtDNAs, together with the observation that the African sequence is most diverged among humans, strongly supports the recent African origin of modern humans, *H. sapiens sapiens*.

B-51

AN ASSOCIATION BETWEEN THE ANGIOTENSINOGEN GENE POLYMORPHISM AND THE BLOOD PRESSURE IN JAPANESE. Ying Ning ZHANG (Dept. Health Sciences, Yamanashi Med. Univ.), Zentaro YAMAGATA (Health Care Center, Yamanashi Univ.), Toshihiro MIYAMURA, Shininchi SHINOZAKI, Sumio IIJIMA and Akio ASAKA (Dept. Health Sciences, Yamanashi Med. Univ.)

We studied on an association between the angiotensinogen (AGT) gene polymorphism and the blood pressure on 317 healthy unrelated Japanese males, aged from 26 to 72 years(mean=39.7 ± 9.1). We observed AGT genotype using the mismatch PCR method which detected Met235-Thr235 allele in AGT gene. This study showed that the number of individuals with genotype MM was 21 (6.6 %), MT 110 (34.7 %) and TT 186 (58.7 %). The genotype frequency was thus 24.0 % for M and 76.0 % for T. It indicates clear racial differences. This population was in Hardy-Weinberg equilibrium. The genotype frequency was 0% for MM, 30% for MT and 70% for TT in 20 individuals affected with hypertension, though 7% for MM, 35.0% for MT and 57.9% for TT in 297 unaffected. There was no association between AGT genotype and blood pressure in this study.

B-52

Association between deletion polymorphism of angiotensin-converting enzyme gene and myocardial infarction in the Japanese population. Liming LI, Shuichi KIKUCHI, Tadao ARINAMI, Kimiko KOBAYASHI, Hideo HAMAGUCHI (Dept.Med.Genet.Univ.Tsukuba)

A deletion polymorphism in the gene encoding angiotensin-converting enzyme is related to the level of circulating enzyme (Rigat et al.1990). It has been reported that the D allele of the polymorphism is associated with myocardial infarction (MI) especially in people otherwise classified as low risk (non-obese subjects with normal lipid values),and with a parental history of MI in the Caucasian (Cambien et al.1992). We have investigated the relationship between ACE gene polymorphism and MI in 459 unrelated healthy Japanese individuals and 185 cases with MI. The genotype distribution was 15% and 12% in DD, 63% and 45% in DI, and 23% and 44% in II in 41 cases with low risk (total cho<200mg/dl and body mass index<23.5kg/m^2) and 459 controls, respectively. The difference in the sum of the DD and DI was significant (p=0.007). The similar association of DD and DI with MI was observed in 118 non-diabetes cases. The data suggest that the ACE deletion polymorphism may be an important risk factor for MI in non-obese subjects with normal lipid levels and in non-diabetes subjects in Japanese.

B-53

AN ASSOCIATION BETWEEN THE VITAMIN D RECEPTOR GENE POLYMORPHISM AND THE BONE MINERAL DENSITY. Zentaro YAMAGATA (Health Care Center, Yamanashi Univ.), T.MIYAMURA, Y.ZHANG, S.SHINOZAKI, S.IIJIMA and A.ASAKA (Dept. Health Sciences, Yamanashi Med. Univ.)

We studied on an association between the vitamin D receptor(VDR) gene polymorphism and the bone mineral density(BMD) on 102 healthy unrelated Japanese females, aged from 23 to 68 years.This study showed that the number of individuals with genotype bb was 56 (54.9 %), Bb 39 (38.2 %) and BB 7 (6.9 %). The gene frequency was thus 74.0 % for b and 26.0 % for B. It indicates clear racial differences. BMD's Z score values of total subjects increased in the following order: from individuals with genotype BB, through those with genotype Bb to those with genotype bb.The allele b was associated with the higher BMD in the Japanese. The BMD decreasing rate increased in the following order: from individuals with genotype bb, through those with genotype Bb to those with genotype BB. This result indicates VDR gene polymorphism is associated with the BMD decreasing rate, too. Our study showed that this gene polymorphism has an effect on BMD regardless of race.

B-54

THREE CASES OF ISOCHROMOSOME FOR THE SHORT ARM OF THE Y i(Yp). Mashio KITATANI, Mamoru OZAKI, Hiroaki TAKAHASHI(Clini. Genet., Dept of Hum. Genet., Med. Res. Inst., Kanazawa Med. Univ. Ishikawa), Hisaya KUNIBE, Haruo TAKABAYASI, Soryu KUWABARA(Dept. Obstet. Gynecol., Kanazawa Med. Univ., Ishikawa), Hideki KAWAGUTI, Tohru KANZAKI(Dept of Perinat., National Crdiovascular center, Osaka)

Three patients with 45,X/46,X,+mar were investigated by cytogenetic and molecular analysis. Case 1 was a male fetus (30 weeks' gestation) referred for karyotype analysis following the observation of oligoamnios and polycystic kidney at the ultrasound scan. Fetal blood cell culture confirmed the 45,X/46,X,+mar/47,X,+mar,+mar karyotype. Case 2 was a infant (17 days of age) with clitomegary and inguinal hernia. Peripheral blood cell culture confirmed the 45,X/46,X,+mar/47,X,+mar,+mar karyotype. Case 3 was a 28-year old woman with gonadal dysgenesis. Peripheral blood cell culture confirmed the 45,X/46,X,+mar karyotype. In all patients the marker chromosomes had the size of G chromosomes, yet failed to show both the characteristic bright fluorescence and C-band. The maker chromosomes were seemed to be pseudodicentric chromosomes with duplication of Yp and euchromatic Yq sequences by using G-band. Polymerase chain reaction demonstrated that the marker chromosome was of Yp origin in case 2.

B-55

CYTOGENETIC AND MOLECULAR ANALYSIS OF TWO TURNER SYNDROME PATIENTS WITH MARKERS OF Y ORIGIN. **Sahar M. GAMAL, Tomoko HASEGAWA, Masako KONDO, Hiroshi MINAMI, Minoru HAMAZAKI, Koichi ENDO** (Shizuoka Children's hosp.), **Hitoshi SATOH** (Tokyo Inst. Med. Sci), **Yutaka NAKAHORI and Yasuo NAKAGOME**(School Int'l Health, Tokyo Univ.).

Two cases of Turner syndrome showing non-fluorescent marker chromosomes in their cultured blood lymphocytes are reported. FISH was performed using Y cocktail probe (DYZ1/DYZ3) and proved the Y origin of the marker in both cases. PCR analysis showed partial deletion of Yq of the first patient. Gonadectomy was performed for the possibility of gonadoblastoma formation. Postoperative pathological examination of the removed gonads revealed bilateral streak gonads in the first patient and gonadoblastoma tissue in the second. FISH study was also performed on buccal smears and gonadoblastoma tissue of the second patient and positive signals showed mosaic distribution of the marker in both tissues at a frequency of 54.1 and 65.3% respectively. This study revealed the presence of the Y-containing cells in a mosaic distribution in the gonadoblastoma tissue of a Turner syndrome patient using FISH.

B-56

CONFIRMATION OF THE CHROMOSOMAL REGION CRITICAL FOR DOWN SYNDROME (DS) BY FISH ANALYSIS IN A DS PATIENT WITH 21p+

Naomichi MATSUMOTO, Tohru OHTA, Hidenobu SOEJIMA, Tatsuya KISHINO, Yoshihiro JINNO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Makoto MIKAWA, Nobuyoshi ISHIKAWA (Dept. Pediat., Kitami Red Cross Hosp., Kitami)

A female infant with clinical Down syndrome (DS) was found to have a de novo mos46,XX/46,XX,21p+ karyotype. Derivation of the additional material on the short-arm of chromosome 21 was examined by fluorescence in situ hybridization (FISH) using 3 chromosome 21-specific probes: CGA2G07 mapped to 21q11,2, CHCS to 21q22.1, and CRI-L427 to 21q22.3. Since the patient suffers from DS, we predicted that the extra-material would have been derived from a distal segment of chromosome 21 involving its q22.3 region that is responsible for most of the DS manifestations. FISH analysis revealed that the marker chromosome 21 is composed of a normal # 21 and a segment from at least 21q22.1 to 21q22.3. The karyotype is thus interpreted as mos46,XX/46,XX,der(21)t(21;21)(21qter→ 21q22.1::21p11.2→ 21qter). The result confirmed the critical region of Down syndrome.

B-57

TWO UNRELATED CASES OF PARTIAL dup(3) MOSAICISM.

Mitsuo MASUNO, Yoshio MAKITA, Mihoko NAKAMURA, Kiyoshi IMAIZUMI, Yoshikazu KUROKI (Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama)

Case 1 The patient was 8-year-old boy. He had dysmorphic face including hypertelorism, scant medial eyebrows, flat nasal bridge, thick upper lip, and full cheeks, growth retardation, and psychomotor developmental delay. His karyotype was 46,XY/46,XY,dir dup(3)(p21.31→p25.3) [17:20]. The chromosome 3 origin of the duplicated material was confirmed by FISH, using a whole chromosome 3 painting probe (Oncor). Two color FISH analysis using D3S671 (3p22) and D3S961 (3p24.2) [kindly provided by Dr. Y. Nakamura] confirmed direct duplication.

Case 2 The patient was 4-year-old boy. He had mental retardation and dysmorphic face including frontal bossing, esotropia, scant medial eyebrows, low nasal bridge, full cheeks, and narrow palate. His karyotype was 46,XY/46,XY,inv dup(3)(p25→p23) [10:20]. The chromosome 3 origin of the duplicated material was confirmed by FISH, using a whole chromosome 3 painting probe. However, two color FISH analysis using D3S671 (3p22) and D3S961 (3p24.2) did not show duplication of the two probes. Further analysis using other cosmid probes on the chromosome 3 is now under way.

B-58

CONFIRMATION OF PARTIAL TRISOMY 47,XY,+der(13)t(9;13)(p11;q11)mat BY USING PCR METHOD.

Yogo HARAGUCHI, Akio FURUSE, Mikihiko MURAKAMI (Kumamoto-chuo Hospital), Masaki TAKIGUCHI, Masataka MORI (Dept. Mol. Genet. Kumamoto Univ.), Fumio ENDO and Ichiro MATSUDA (Dept. Pediatr. Kumamoto Univ.)

A moderately mental and developmental retarded boy with partial trisomy 47,XY,+der(13)t(9;13)(p11;q11)mat was reported. His clinical findings were similar to those of trisomy 9p patients which included microcephaly, low-set ears, globulous nose, ocular hypertelorism, clinodactyly and joint abnormalities. Attempts to confirm the partial trisomy were carried out by using PCR at three dinucleotide repeat polymorphisms localized on 9p21, 9p13 and 13p-13q12, of which locus is RPS6, D9S200 and D13S192, respectively. The analysis of family members with RPS6 polymorphism demonstrated that the patient had trisomy of 9p21, as showing three main bands derived from two of mother and one of father on natural polyacrylamide gel electrophoresis. This prompt method of PCR may facilitate screeing and/or confirmation of other trisomy cases.

B-59

CASES WHERE FISH TECHNIQUE WAS EFFECTIVE IN IDENTIFICATION OF CHROMOSOMAL ABNORMALITIES.

Reiko YAMASHITA, Yoshito TSURUKUBO, Hiroshi IKARASHI, Kazuo SUWA (BML, Tokyo), and Kiyomi YAMADA (Nat Med Cent Res Inst, Div of Genetics, Tokyo)

Since there are limitations in the identification method by characteristics of banding patterns on chromosomes even if various banding techniques are used, we occasionally have encountered difficulty to identify structural abnormalities of chromosomes, the origin of extra band material or marker chromosomes during routine chromosome examination in patients with congenital diseases. For identification of undetermined abnormalities, we have recently tried to use the FISH method using chromosome-site specific or painting DNA probes. We demonstrated here 5 cases that we could obtain conclusive findings on undetermined abnormalities by application of the FISH method: In three cases of markers, a minute marker of pericentromeric region was identified as X and Y origin, respectively, and one extra marker was identified as idic(15). A microdeletion of 15q11-13 region was determined using disease-specific probes in a patient with Angelman syndrome, and de novo t(5;18) was identified in a 5p+ case. We reported that the FISH method was convenient and powerful technique necessary for precise identification, and should be applied to chromosome examination in such congenital cases that chromosome abnormalities were not determined inspite vigorous analysis using various banding techniques.

B-60

APPARENT MONOSOMY 21:RULED OUT BY CHROMOSOMAL PAINTING FISH (FLUORESCENCE IN SITU HYBRIDIZATION). Keiko IWASAKI, Sahar M GAMAL, Tomoko HASEGAWA (Div. Clin. Genet. Shizuoka Children's Hospital, Shizuoka) Takeyasu IGARASHI (Div. Neonatology. S. C. H., Shizuoka), Kouichi ENDO, Motohiro IKUTA (Div. Clin. Path. S. C. H., Shizuoka)

Recently, cases of apparent monosomy 21 have been demonstrated to have translocation by new methods such as FISH. We examined a newborn female infant with multiple anomalies, the second child of a 31-year-old father and a 35-year-old mother. Cleft palate, micrognathia, deformed pinnas, low-set ears, imperforate anus, over-lapping fingers, rocker-bottom feet, arthrogryposis, and bilateral congenital dislocation of the hip were seen. Since monosomies other than those of sex chromosomes are thought to be incompatible with survival, we thought that the patient might have translocation of chromosome 21, and therefore further studies were done despite the apparent 21 monosomy revealed by G-band staining. Three signals were seen on all cells in peripheral blood and buccal smears by FISH using a 13/21 α-satellite probe. Fluorescence of chromosome 21 was seen at the terminal and of the short arm of a C-group chromosome. High resolution G-band staining showed that the 21q21.1-qter was translocated to 8p23.1, and thus the abnormalities of the patient were caused by de novo partial monosomy 8p and 21q.

B-61

Duplication of 8q23 detected by FISH using a cosmid probe. Keiko WAKUI, Akira YAMAGISHI, Hirofumi OHASHI, Shin-ichiro HAMANO, Takahiro NARA, Yusuke NAKAMURA* and Yoshimitsu FUKUSHIMA (Saitama Children's Medical Center and Department of Biochemistry, Cancer Institute*)

The proband was a 6-month-old female infant. She was born at 40 weeks' gestation to a 29y father and a 22y mother, both healthy and non-consanguinous. Her birth weight was 2922g. She was referred to our Center at 1 month, because of her convulsions. Clinical features of the patient were: hypertelorism, thin upper lip, small ears, short neck, wide set nipples, hemangioma over the left eyelid and ear, and mild mental retardation. She was diagnosed as having epilepsy with abnormal EEG. Chromosome analysis by G-banding revealed that she had an excess dark-band on 8q distal region. The parents had normal karyotypes. As the excess band was likely to be 8q23, we performed FISH analysis using a cosmid probe mapped on 8q23 (cCI8-1173). We detected 1 pair of signals of the probe on one chromosome 8 and 2 pairs on the other chromosome 8. The patient was proved 8q duplication including 8q23 region.

It is difficult to identify a small excess chromosome band except for the cases of derivative chromosome from the parental balanced translocation. Even by the microdissection-chromosome painting method, it is hard to detect a small interstitial excess band such as this case. FISH analysis using a mapped cosmid probe is very useful for identification of constitutional chromosome aberrations. We hope for clinical applications of these techniques and the establishment of the cytogenetics-molecular genetics collaboration system.

B-62

A BOY WITH PRADER-WILLI SYNDROME AND HIS CLINICALLY NORMAL MOTHER, BOTH WITH DUPLICATION OF THE PWS/AS REGION: FISH AND CA REPEAT ANALYSIS.

Takazumi KOZAKI, Saori HABUTA, Keiko TAKAHASHI, Hideyuki UMII, Yoshimori ISHIHARA, Tadashi KAJII (Chromosome Analysis Laboratory, SRL, Inc.), Akira KUWANO, Ikuko KONDO (Dept. Hygiene, Ehime Univ.), Takako TERANISHI and Masami YAMATANI (Dept. Pediatr. Toyama Kyoritu General Hospital)

A 9-year-old boy showed typical clinical manifestations of PWS: infantile hypotonia, mental retardation, obesity, pale skin, a small nose, small hands and feet and a small penis. The boy and his clinically normal mother both showed duplication of the 15q11.2 segment, spanning both the PWS and AS critical regions,as evidenced by chromosomal FISH (D15S11,SNRPN,D15S10 and GABRB3) and analysis of CA repeat polymorphisms(D15S11,GABRB3 and c-fes). His clinically normal sister was without the duplication, while the father was unavailable for study. Uniparental disomy of chromosome 15 in the boy was ruled out based on the CA repeat analysis. The duplicated segments were nonidentical both in the mother and son, an indication that the duplication resulted from unequal crossing over between homologous chromosomes 15 in an earlier generation.

B-63

ANALYSIS OF WILLIAMS SYNDROME BY FLUORESCENT *IN SITU* HYBRIDIZATION

Hamao HIROTA, Atsuyoshi TAKAO, Misa KIMURA, Shin-ichiro IMAMURA, Kazuo MOMMA, Rumiko MATSUOKA (Dept. of Pediatric Cardiology, Tokyo Women's Medical college, Tokyo)

The Williams syndrome (WS), characterized by elfin face, mental and statural deficiency and, often, supravalvular aortic stenosis (SVAS), is caused by mutations in the elastin gene (7q11.23). In order to clarify the phenotype and regional deletion correlation, 20 WS, and 14 SVAS without elfin face patients, and 20 family members were examined by fluorescent *in situ* hybridization (FISH), using the Williams Syndrome Chromosome Region probe (Oncor). Monosomy for a region of 7q11.23, including an elastin gene, was found in all 20 WS, but was not found in any of the 14 SVAS and 20 family members. These data indicate that deletions involving one elastin allele cause WS and implicate elastin hemizygosity in the pathogenesis of disease. Further investigations are necessary to clarify whether point mutations or very small deletions are present in SVAS without elfin face.

B-64

CATCH 22 SYNDROME: PHENOTYPE AND del 22q11.2 CORRELATION.

Rumiko MATSUOKA, Atsuyoshi TAKAO, Misa KIMURA, Shin-ichiro IMAMURA, Masahiko ANDO, Kazuo MOMMA (Tokyo Women's Medical College, Tokyo) and Kunitaka JOH-O (Kyushu Welfare Pension Hospital, Fukuoka).

The CATCH 22 syndrome, Cardiac defects, Abnormal faces, Thymic hypoplasia, Cleft palate, and Hypocalcemia resulting from 22q11.2 deletions, including conotruncal anomaly face syndrome (CTAFS) and DiGeorge syndrome (DGS). To clarify the phenotype and del 22q11.2 correlation, 88 CTAFS patients and 10 DGS patients, 17 parent couples, 20 mothers and 3 fathers were examined by fluorescent *in situ* hybridization using a N25 (D22S75) DGCR probe. Monosomy for a region of 22q11.2 was found in 80 CTAFS, 8 DGS, 6 mothers and 1 father. For the control, 126 patients who had cardiac defects without CTAFS were examined and had no deletion of 22q11.2. Cardiac defects most frequently associated in 88 del 22q11.2 patients were tetralogy of Fallot (64/88, 72.7%, especially with aortic arch and neck vessels anomalies 57/64, 89%).

B-65

HYPOMELANOSIS OF ITO ASSOCIATED WITH X;15 TRANSLOCATION.

Ken-ichi OHBA, Tohru SONODA, Tohru SUGIMOTO (Dept Ped., Miyazaki Med Coll, Miyazaki), Shozo OHDO (Dept Ped, Natl Sanatorium Miyazaki Hosp, Miyazaki) and Kiyomi YAMADA (Devi Genet, Clin Res Inst, Natl Med Cent Hosp, Tokyo)

We report on a girl with hypomelanosis of Ito(HI). She has typical skin lesion and neurologic impairment consisting of hypotonia and developmental retardation. Cytogenetic investigation showed a X/15 translocation with breakpoint on Xp11.23 or 11.21. HI is considered a clinical manifestation of chromosome mosaicism or chimerism but several X-autosome translocation involving Xp11 have been reported. Our patient has also the same breakpoint. X chromosome rearrangements have been detected in sporadic cases of Incontinentia Pigmenti (IP1), with most of the breakpoints within Xp11. Differentiation between these two conditions is not easy. Some sporadic cases of IP due to X-autosome translocation at Xp11 have no history of blistering and clinical feature is atypical. We present further evidence that one of the genetic forms of HI is localized at Xp11 and also discuss clinical features of X-autosome translocation involving Xp11.

B-66

DETECTION OF ANEUPLIDY IN HUMAN SPERMATOZOA USING MULTI-COLOR FLUORESCENCE IN SITU HYBRIDIZATION. Osamu SAMURA, Norio MIHARU, Satoshi HAYASHI, Hu He, Koso OHAMA (Dept. OB/GYN., Hiroshima Univ. of Med., Hiroshima)

We applied multi-color FISH to study the frequency of disomy and diploidy in spermatozoa. Semen samples were obtained from 5 healthy men. To make sperm nuclei penetrable for DNA hybridization, we pretreated the semen slides with dithiothreitol and lithium diiode-salicylate. We utilized alpha-satellite DNA probes (Oncor Inc.) for chromosome 1 (D1Z2 ; biotin), 16 (D16Z2 ; biotin),18 (D18Z1 ; biotin and digoxigenin),X (DXZ1 ; digoxigenin) and Y(DYZ3 ; biotin) analyzing a minimum of 6000 sperm nuclei for each probe with each sample. The frequencies of disomic nuclei were : 0.23% (0.19-0.31%) for chromosome 1, 0.11% (0.06-0.15%) for chromosome 16, 0.14%(0.09-0.23%) for chromosome 18, 0.08% (0.05-0.13%) for X-X, and 0.10% (0.06-0.14%) for X-Y, 0.09% (0.05-0.21%) for Y-Yand the diploid frequencies was 0.15%(0.12-0.18%). This method proved to be useful for determining aneuploidy of human chromosomes in spermatozoa and may be valuable for studies of male non-disjunction.

B-67

ANALYSIS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX CLASSⅢ REGION BY HIGH RESOLUTION FLUORESCENCE IN SITU HYBRIDIZATION
Yumiko SUTO, Masumi OHTA, Momoki HIRAI (Dept. Anthropol., Grad. Sch. Sci., Univ. Tokyo, Tokyo), Yoshihisa WATANABE and Katsushi TOKUNAGA (Dept. Res. Japan. Red Cross Central Blood Center, Tokyo)

Visual mapping of stretched DNA by fluorescence in situ hybridization (FISH) is a new physical mapping technology. The use of this technique allows the rapid mapping of DNA probes with less than 10 kb resolution. We analyzed the gene organization of the MHC class Ⅲ region by this technique. Biotinylated C4 cDNA and digoxigenin-labeled 21OH cDNA were hybridized to decondensed nuclei from individuals with different C4/21OH haplotypes, and hybridization signals were detected with FITC and rhodamine, respectively. Each cell with normal haplotype showed two tandem arrays of green and red signal clusters (C4A-21OHA-C4B-21OHB). Gene duplication was visualized as addition of the signal cluster. Gene organization determined by this method was in good agreement with the results previously obtained by Southern blot hybridization.

B-68

DOSE-RESPONSE RELATIONSHIP FOR STABLE CHROMOSOME ABERRATION FREQUENCIES IN LYMPHOCYTES OF A-BOMB SURVIVORS. Akio A. AWA
(Radiation Effects Research Foundation, Hiroshima)

Stable chromosome aberrations (mainly reciprocal translocations and inversions) are known to persist for decades in the peripheral blood lymphocytes of A-bomb survivors. This report describes the results of chromosome aberration analysis by conventional, G-band and FISH methods on survivors in the RERF Adult Health Study cohort. Major findings have shown that:

(1) A positive dose-response relationship has been observed for frequencies of stable chromosome aberrations, although there is a difference in aberration frequencies between the two cities; the dose-response curve is linear in Hiroshima, whereas the dose-square term is much greater in Nagasaki. For all dose ranges, the aberration frequency is consistently higher in Hiroshima than in Nagasaki. (2) There is a wide variability of the frequency of stable chromosome aberrations between survivors in a given dose range. When the data are limited to survivors who were irradiated inside the Japanese-type house at exposure, the observed variability in the distribution of aberration frequencies has become smaller. A city-difference noted previously has also become markedly reduced, when the data on the Japanese-house survivors are used exclusively.

B-69

The Homologies at DNA level between human and mammalian chromosomes

Fang-Yang WU, Kumiko IIJIMA, Makoto HIGURASHI
(Department of Maternal and Child Health, School of Health Sciences, Faculty of Medicine, University of Tokyo, Tokyo)

We present an approach for analyzing chromosome homologies in the mammalian evolution by the use of CISS hybridization with human chromosomes 4- and 6-specific DNA libraries. In chimpanzee and macaque, which demonstrates that homologies of human chromosome 4 and 6 DNA content parallels the homologies of chromosome morphology. In gibbon, whose chromosomes can not be identified as homologous to the human chromosome 4 and 6 based on G-banding patterns, but CISS hybridization had overcome this difficulty. Human chromosome 4 and 6 DNA libraries stained five different chromosome pairs. Our results showed a high evolutionary conservation in Pongidae and Cercopithecidae. In lower primates and the domestic cats, the capuchin chromosome 4 were labeled by human chromosome 6 DNA libraries and showed complete homology to human chromosome 6, however, the capuchin chromosome was not labeled by human chromosome 4 DNA libraries. Lemur and the domestic cat chromosomes were not labeled by human chromosome 4- or 6-specific DNA libraries.

C-1

EXTENSIVE MONGOLIAN SPOTS IN THREE PATIENTS WITH METABOLIC DISORDERS. Hirofumi OHASHI, Masaki SHIMIZU, Takahiro NARA, Naoya KODA, Shuichi YAMAGUCHI, Kazuko OBANA, Satohiko IMAIZUMI, Toshie MATSUSHIMA, Eisaku SATO, Hirofumi KIMOTO, Tsutomu OHNO, Kibo YOSHIDA, Yoshimitsu FUKUSHIMA (Saitama Children's Medical Center)

Mongolian spots (MS), congenital blue macular lesions due to dermal melanocytosis most commonly seen around the presacral area, are observed in more than 80% of oriental infants and usually disappear by around seven years of age. MS have been generally considered not to be associated with specific disorders. We report on a boy and twin brothers in whom both extensive MS and a metabolic disorder were present. Patient 1 is a 2-year-old boy with GM2-gangliosidosis, and Patient 2 and 3 are 17-month-old twin brothers with Hunter disease. All these three patients were noticed to have extensive MS over the back, buttock, and posterior aspects of the thigh. Mendez et al. (AJMG 47:456-457, 1993) suggested that extensive MS was causally associated with metabolic disorders. At least 20 patients with Hurler disease and 4 with GM1 gangliosidosis have been reported as having extensive MS. Present patients might support the possibility that extensive MS is a sign of some metabolic disorders, including not only Hurler disease and GM1-gangliosidosis but also Hunter disease and GM2-gangliosidosis.

C-2

A CASE OF TORIELLO-CAREY SYNDROME. Michiko NONAKA (Dept. Medical Genetics, Kobe Children's Hospital, Kobe)

We describe a 2 year-old-boy with multiple congenital anomalies, we think, in another case of the Toriello-Carey syndrome. This boy was the second child, born at term, weighed 3048g, to a 30 year-old gravida 2, para2, Japanese mother and a 32 year-old Japanese father, following normal pregnancy that was uncomplicated by known teratogenic exposure. There was no parental consanguinity or family history of congenital abnormalities. The main manifestations include unusual face appearance with Robin sequence, bilateral ear deformity with hearing loss, excess neck skin, hypoplasia of the corpus callosum, optic nerve anomaly (morning glory syndrome), laryngeal dysplasia, cardiac defect (ECD, Ebstein's anomaly) and severe post-natal growth retardation with hypotonia, in the absence of known chromosome defects.
After Toriello and Carey reported (Am J Med Genet, 1988) on four infants with a multiple congenital anomaly (MCA) syndrome, eight similar cases were reported. To our knowledge, no patients with this condition have been reported in Japan.

C-3

FATHER AND SON WITH GREIG CEPHALOPOLYSYNDACTYLY SYNDROME (MIM No. *175700): HIGHLY VARIABLE EXPRESSIVITY IN A FAMILY

Satoshi ISHIKIRIYAMA, Reiko NEMOTO, Chiaki ITOH, Hiroaki DATE, Ryohko SHIMAMOTO, Akikazu UDAGAWA (Chiba Cildren's Hospital, Chiba)

A male baby was born to healthy, unconsanguineous parents after an uneventful pregnancy. At birth, his occipitofrontal circumference (OFC) was 33 cm (-0.43 SD). He had not only preaxial and postaxial polydactyly with syndactyly on the hand but also preaxial polydactyly with syndactyly on the foot, bilaterally. At one month old, he had macrocephaly (OFC; 42cm, +3.3 SD) and an expanded frontal fontanel. On CT scan, enlarged lateral ventricles were found. Afterward, the head did not extraordinarily enlarge. His growth and developmental milestones were within normal limits. His father had only very broad thumbs.

The son was one of the most severely affected ptients with Greig cephalopolysyndactyly syndrome (GCPS), while his father was one of the least severely affected ones with GCPS. This exhibited one of the most highly variable expressibity in a family. It is important to carefully examine thumbs at genetic counseling about GCPS. A putative gene for GCPS (GLI3) was recently isolated. It might be useful to detect mildly affected ones with GCPS by a molecular biological method. Prenatal diagnosis by ultrasonography is more practical than that by a molecular biological method, because parents want to know not whether a fetus has a GCPS allele but how severely a fetus affected with GCPS.

C-4

TWO CASES WITH LIMB - GIRDLE MUSCULAR DYSTROPHY SHOWING A POSTURE WITH A NECK DROPPED BACKWARD AT WALKING.

Katsuhito ADACHI, Chiyomi KIMURA, Takako NARUO, Setsuko KASHIWAGI, Toshio INUI (Dept. Internal Med., Natl. Sanatorium. Tokushima Hosp., Tokushima) and Hisaomi KAWAI, Takao MITSUI, Makoto KUNISHIGE, Yoshihiko NISHIDA (Dept. First Internal Med., The Univ. of Tokushima , School of Med., Tokushima)

We reported two cases with limb - girdle muscular dystrophy showing a posture of which they support their occipital region by their left hands. Case 1 is a 22 - year - old male. His elder sister showed similar muscular atrophy. His initial gait was observed at one year and a half. He began to walk slowly at 3 years of age, and climed up himself at 9 years. Case 2 is a 20 - year - old male. His parents were consanguineous. His initial gait was observed at 2 years and a half. He climed up himself at this age. He could go up and down the stairs by a rail untill 15 years. They have predominant muscular atrophy at neck and proximal regions of the four limbs, and have pseudohypertrophy at gastrocnemius muscles. They walked supporting their occipital region by their left hands. Serum CK activities of both cases are as high as 1,897, 2,805 IU/L, respectively. Dystrophin gene deletion was not detected by PCR method. Immunohistochemistry of the muscle tissues showed degenerations of muscle fibers, and showed normal staining of dystrophin, utrophin, and 43kDa dystrophin associated glycoprotein (DAG), but weak staining of 50kDa DAG in case 2. The present cases with predominant limb - girdle muscular atrophy have an unique posture with neck dropped backward caused by weakness of the flexor neck muscles. They may be classified as a subtype of limb - girdle muscular dystrophy.

C-5

UMBILICAL FINDINGS IN AARSKOG SYNDROME. Masato TSUKAHARA, Susumu FURUKAWA(Dept. Pediatr. Yamaguchi Univ. Sch. Med., Yamaguchi)

We report on five patients with Aarskog syndrome who represent undescribed features of the umbilicus. Of the five patients, two had protruding umbilicus, while the other three had the characteristic umbilicus consisting of the smooth depression with radiated branches of the cicatrix, and flat cushion. These umbilical configurations associated with Aarskog syndrome have not been described previously. The flat configuration of the umbilicus could also be the characteristic umbilical finding associated with Aarskog syndrome as well as the protruding umbilicus.

C-6

ANALYSIS OF SERUM URIC ACID LEVELS IN PATIENTS WITH DOWN SYNDROME. Yoshitaka SHIBUYA[1,3], Hidefumi TONOKI[2], Toshio TAKAKUWA[3] ([1]Dept. Pediatr., Shinnittetsu Muroran General Hospital, Muroran: [2]Dept. Pediatr., Hokkaido Univ., Sapporo: [3]Taiyonosono Training Center, Date)

The elevation of serum uric acid (UA) levels in patients with Down syndrome (DS) has been reported by several investigators, but gout or renal dysfunction due to hyperuricemia has rarely been encountered. However, there has been no statistical report of hyperuricemia in patients with DS in Japan. We measured serum UA levels of 48 patients with DS (34 male; age 17 to 44, 14 female; age 18 to 44) living at Taiyonosono Training Center. The controls were selected and matched for age and sex among other mental retarded patients at the Center. The average of serum UA levels in male patients with DS was 7.2 mg/dl, which is significantly higher ($p<0.001$) than that in the controls (5.2 mg/dl) as well as in female (5.1 mg/dl in DS vs. 4.1 mg/dl in the controls, $p<0.05$). Statistical analysis revealed that serum UA levels were related significantly to obesity in male patients with DS. This is the first report of analysis of serum UA levels in Japanese patients with DS. Although, no DS patient in the Center is symptomatic of hyperuricemia, further investigation seems to be necessary in Japan.

C-7

AN ANTHROPOMETRIC STUDY ON THE GROWTH IN 7 CASES OF CRI DU CHAT SYNDROME.

Chun Man PARK, Kumiko IIJIMA, Makoto HIGURASHI (Dept.MCH, Health Sciences, Faculty of Medicine, Univ. of Tokyo, Tokyo)

Cri du chat syndrome is resulted from partial delition of the short arm of chromosome 5. It has been named out of the resemblance of the cry to the mewing of a cat. Growth and development are always retarded. We report here that we have been observed growth and development of 7 cases (6 boys, 1 girl) with this syndrome, longitudinally. Ages ranged from 3 years to 18 years. At birth the average of weight, height, head circumference, chest circumference and gestation period were 2705g, 47.2cm, 32.8cm, 31.6cm and 39.2 weeks respectively. One case was suffered from not only cnngenital heart disease (CHD) but also polycystic malformation. Concerning gross motor head control was performed at age 3 to 6 months. Rolling 3 to 6 months. Crawling 8 to 18 months. Sitting 7 to 24 months. Walking 18 months to 4 years with the exception of 2 unwalkable cases whose ages were 4 yr and 18 yr. Most cases were before the onset of puberty. Their height and weight from -1SD to -2SD, and head circumference under -2SD. Only one case, thought to reach final height, was severely retarded. His height, weight and head circumference were 142.7cm, 33Kg and 48.8cm respectively. These were under -3SD.

C-8

VERTUES OF A FAMILY BOWLING MEETING FOR YOUTHES WITH DOWN SYNDROME.

Hiroshi NAKAI, Hitoshi MIKAMI, Koichiro IKE, Toshio OHARA (Dept. Pediatr.,Iwate Prefectural Central Hospital, Morioka)

To make activities of handicapped people higher and to have times being with non-handicapped people for them more, we had a meeting together to play bowling.

The plan was made after a lecture about interaction among patients with Down syndrome and non-handicapped teenagers, which was reported by Rynders J.E. et al. in a paper on Am. J. Ment. Def.,85:268-273,1980.

Almost of attendants with Down syndrome had never experience of bowling play but their family could teach them how to play it. After several advices and training, they could enjoy the play. They could express their mind and thinking well by their words or movements. They always understand results of their throwing bowls one by one and express their impression. They also sometimes appriciated plays of other members and shared their joy of success or regret of missed play to non-handicapped people. These actions will improve not only physical condition but also mental status of the player with Down syndrome. This will also prevent adult diseases such as obesity, hyperlipidosis, diabetes millitus, heart diseases etc. It is needed that more members can join the meeting together.

C-9

GENETIC LINKAGE ANALYSES OF ROMANO-WARD SYNDROME IN 13 JAPANESE FAMILIES.
Toshihiro TANAKA (Dept.Biochem.,Cancer Institute, Tokyo, 3rd Dept.Int.Med., Univ.Tokyo, Tokyo), Yoshimitsu FUKUSHIMA (Div.Genetics, Saitama Children's Medical Center, Saitama), Ken-ichi NAKAHARA, Tsutomu YAMAZAKI, Ryozo NAGAI, Yoshio YAZAKI (3rd Dept.Int.Med., Univ.Tokyo, Tokyo) and Yusuke NAKAMURA (Dept.Biochem.,Cancer Institute, Tokyo)

Romano-Ward syndrome (RWS) is an autosomal dominant disorder characterized by prolongation of the electrocardiographic QT interval, with clinical manifestations that include recurrent syncope and sudden death from ventricular arrhythmias. In the previous studies, *HRAS* was reported to be tightly linked to LQT locus in one ethnic group, while in another, it was not linked at all. To find an LQT locus in Japanese patients, linkage analyses were undertaken in 13 families with RWS patients with two DNA markers (*D11S922* and *HRAS*) located on 11p15.5. In our analysis, overall maximum lod score at *D11S922* was 3.91 at θ = 0.11. Analysis of homogeneity suggested genetic heterogeneity of RWS within the Japanese population.

C-10

GENETIC SUSCEPTIBILITY FOR PARKINSON'S DISEASE.
Ikuko KONDO, Yuji MORIMOTO,Akira KUWANO(Dept. Hygine, Ehime Univ. Sch.Med., Ehime) Yoriaki YAMASHITA(Dept.Neurol., Matsuyama Red Cross Hosp., Ehime) Ichiro KANAZAWA(Dept. Neurol. Tokyo Univ.,Tokyo) and Yoshikuni MIZUNO(Dept. Neurol. Juntendo Univ. Sch. Med.,Tokyo)

Pathogenesis of Parkinson's disease(PD) is likely to be multifactorial, deriving from environmental factors acting upon genetically predisposed individuals. The polymorphic allele of the monoamine oxidase B(MAO-B) gene detected by polymerase chain reaction(PCR) and singe stranded conformation polymorphism (SSCP) was associated with PD in Caucasians. We characterized this polymorphic allele, the allele 1, of the MAO-B gene using direct sequencing of PCR products. A single base substitution (G-A), resulting gain of MaeIII restriction site was detected in the intron 13 of the MAO-B gene. In healthy Japanese controls, the allele 1of the MAO-B gene was twice as frequent as in Caucasian controls. However, the association between the MAO-B gene and PD was not observed in Japanese.

C-11

ASSOCIATION OF A VARIANT OF ANGIOTENSINOGEN GENE WITH PREGNANCY INDUCED HYPERTENSION IN THE JAPANESE. Gen KOBASHI[1], Akira HATA[1], Seiichiro FUJIMOTO[2], Kiyotaro KONDO[1] ([1]Dept. Public Health, [2]Dept. Obstet. Gynecol., Hokkaido Univ. School of Med., Sapporo)

An association between pregnancy induced hypertension (PIH) and a molecular variant in the angiotensinogen (AGT) gene, which encodes methionine (M235) or threonine (T235) at residue 235, was reported both in Caucasians and the Japanese. In Caucasians, T235 was associated with preeclamptic primiparas (PE-PP), a diagnostic subgroup of PIH. However, in the Japanese, an association of T235 with PE-PP is not yet proven probably because of small sample size. To investigate this point, we collected 146 samples of PIH and 292 age and parity matched controls in Hokkaido area. Frequencies of homozygote of T235 were significantly higher in PIH (77%, $p<0.001$), severe PIH (78%, $p<0.001$) and PE-PP (80%, $p<0.001$) than in the controls (55%). In PE-PP, after partitioning the data by severity, association remained significant only in severe type (86%, $p<0.001$), suggesting that severe PE-PP is a more homogeneous entity. It appeared that AGT is involved in the pathogenesis of both PIH and PE-PP in the Japanese.

C-12

CONTRIBUTION OF ANGIOTENSINOGEN TO ESSENTIAL HYPERTENSION IN A COMMUNITY, TAKASU. Akira HATA[1], Gen KOBASHI[1], Koichi SHIDO[1], Iwao SUGIMURA[2], Kiyotaro KONDO[1] ([1]Dept. Public Health, Hokkaido Univ., [2]Asahikawa Kosei Hospital)

An involvement of angiotensinogen (AGT) in the pathogenesis of essential hypertension (EH) was suggested both in Caucasians and Japanese. To investigate the mechanism with which AGT contribute the onset of EH, we collected and genotyped 1615 blood samples in Takasu, Hokkaido, which is 43% of all the population above 30 year old. AGT genotype was determined with respect to residue 235 variant (M235: methionine, T235: threonine). Of those people, 260 were ascertained as hypertensives (HT) and 688 as normotensives (NT) by means of clinical and laboratory data, which is accumulated by annual physical examination started 1975. We found significant association ($p<0.01$) between HT and NT in terms of frequency of homozygote of T235. After partitioning the data by gender, association remained significant only in males. This group of hypertensives were considered to be the appropriate subjects to study the interaction between environmental factor and AGT genotype in the pathogenesis of EH.

C-13

AN ASSOCIATION STUDY ON T235 VARIANT OF ANGIOTENSINOGEN WITH ESSENTIAL HYPERTENSION IN CORONARY HEART DISEASE IN JAPANESE. Hideo HAMAGUCHI, Kimiko KOBAYASHI, Liming LI, Shuichi KIKUCHI, Hiromi HAMADA, Tadao ARINAMI (Dept. Med. Genet., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba)

A common molecular variant of angiotensinogen, T235 (T allele encoding threonine instead of methionine at position 235) has been shown to be associated with essential hypertension and preeclampsia in Salt Lake City, Paris and Japan. Essential hypertension is a major risk factor for coronary heart disease (CHD) in Japanese. We therefore investigated whether T235 is associated with essential hypertension in the patients with CHD whose age of onset of CHD was before 65. The frequency of genotype TT was 0.62 in 256 normotensive healthy subjects (mean age, 53), 0.66 in 315 patients with CHD (mean age, 57), 0.63 in 132 normotensive cases with CHD, and 0.69 in 173 hypertensive cases with CHD, respectively. T235 was not significantly associated with hypertension in CHD. T235 may be a genetic marker which is in linkage disequilibrium with a mutation causing hypertension.

C-14

ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AND CORONARY ARTERY DISEASE IN JAPANESE. Hirofumi NISHI, , Yoshinori KOGA, Tsutomu IMAIZUMI, Hironori TOSHIMA, (Third Dept. Int. Med., School Med., Kurume Univ., Kurume) and Akinori KIMURA, Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

Distribution of the angiotensin converting enzyme (ACE) gene insertion (I) / deletion (D) polymorphism and the plasma ACE activity were investigated in 119 patients with coronary artery disease (CAD). The plasma ACE activity (IU/L) in the CAD patients with DD genotype was 17.4 ± 5.6 which was significantly higher than that in those with II (10.9 ± 3.3) and ID (12.0 ± 4.0) (ANOVA: $p<0.0002$, Scheffe's test $p<0.0001$ for DD versus. II, $p<0.001$ for DD versus. ID). Frequencies of ACE genotypes did not differ between CAD patients and healthy controls (n=234). However, patients having 3 vessel disease (n=23) demonstrated significantly higher frequency of DD genotype (34.8% in the patients and 12.4% in controls, $p<0.02$) with higher plasma ACE level (18.9 ± 7.2 IU/L in the 3 vessel disease group and 16.5 ± 4.6 IU/L in the other patients). These observations suggest that the ACE genotype associates with the plasma ACE activity and severity of CAD.

C-15

CARRIER DIAGNOSIS IN DMD/BMD FAMILIES.
Kayoko SAITO, Reiko MORITA, Akemi YAMAUCHI, Juan DU, Yukiko KAWAKITA, Takayo HARADA, Miyako OGUNI, Kiyoko IKEYA, Eri KONDO, Makiko Osawa, Yukio FUKUYAMA

We analyzed the DMD gene in Duchenne/Becker muscular dystrophy patients and their families, detecting the carrier state. Carrier detection was achieved by examining family pedigree, measuring serum creatine kinase (CK) activity and molecular genetic analysis. In the families with probands in whom partial DMD gene deletions were detected, Southern blot analysis was followed by densitometer scanning to depict the gene dose. Nested-PCR amplification or pulse field gel electrophoresis were also used to confirm the results. Polymorphisms, PCR for pERT87-RFLPs and 3'CA repeats, were examined in the families with or without deletions. A DMD gene deletion was detected in 29 out of 42(69%) families. A maternal DMD gene deletion was detected in 20 of 29(69%) families whose probands showed deletions. The 9 probands(31%) were diagnosed as new mutations, because their mothers showed no deletions. The mothers were confirmed to be carriers in 27 out of 42 families(64%) and to be non-carriers in 9(21%). The carrier risk was determined, based on Bayesian calculations, to be 25-40% in the remaining 6(14%) mothers. Consequently, the maternal carrier/non-carrier state was confirmed in 36 of 42(86%). Twenty-four female relatives other than mothers were determined to be carriers in 3 and to be non-carriers in 15. Composite analysis, by means of family history, CK activity and molecular genetic analysis was thus highly useful for carrier detection.

C-16

A STUDY ON PHENOTYPE-GENOTYPE CORRELATION IN SIX FAMILIES WITH MYOTONIC DYSTROPHY.
Ayako MUTO, Makiko OSAWA, Sawako SUMIDA, Haruko SUZUKI, Yumi ARAI, Keiko SHISHIKURA, Noriko SUZUKI, Kayoko SAITO, Yoshito HIRAYAMA, Reiko YOSHIDA, Makoto FUNATSUKA, Yukio FUKUYAMA, (Dept of Pediatrics, Tokyo Women's Medical College, Tokyo),Tetsuro MIKI (Dept of Geriatrics, Faculty of Medicine, Osaka University,Osaka) and Hiroshi MARUYAMA(Matsudo clinic,Chiba)

Myotonic dystrophy(DM) is the most common inherited muscular dystrophy affecting adults.In 1992, an unstable DNA fragment (a CTG triplet repeat), mapped on chromosome 19q13.3, was isolated and then (1993) named DM kinase. The length of this CTG sequence is known to correlate significantly with clinical severity. The authors conducted a phenotype-genotype crrelation study in six affected(DM) mothers and father, and their respective offspring with DM. Gene analysis was carried out by Southern blot hybridization using EcoRI-cDNA25. DNA analysis revealed an expanded fragment in all cases examined as compared with controls. The length of this fragment ranged from 12.5 to 16.0kb in seven CDM children,from 10.1 to 12.3kb in five DM mothers and one DM child, and 9.8kb in controls. In CDM cases, the presence or absence of a history of hydramnios, neonatal respiratory or feeding difficulty were evaluated later in life and none showed any correlation with the length of the unstable fragment. A significant correlation was found, however, between the age at first clinical manifestation and fragment length. This correlation was well demonstrated in an illustrative pedigree in which a mother and her two sons were affected; mother and younger brother had DM and elder brorher had CDM. Another noteworthy observation was that CDM child whoes mother didn't affected DM has DM father.

C-17

Abnormalities of NF1 gene in patients with Neurofibromatosis 1(NF1).

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

Neurofibromatosis 1(NF1) is one of the most common inherited disorders and characterized by the abnormalities in multiple tissues derived from the neural crest. We screened 50 unrelated Japanese NF1 patients for the mutation of 70% of the coding region of NF1 gene by using polymerase chain reaction(PCR)-single strand conformation polymorphism(SSCP) analysis of exon 28 to 49 and reverse transcriptase(RT)-PCR/SSCP analysis of GTPase activating protein related domain(GRD) and upstream from GRD. These methods revealed 5 deletions, 4 nonsense mutations, one insertion, one silent mutation, one amino acid substitution and 3 splice junction mutations resulting in frameshifts of NF1 cDNA. Half of our mutations were located within exon 30 - 32, including two hot spots, the nonsense mutation in exon 31 for two patients and the splicing alteration in intron 31 for two patients. Our results indicate that the mutation in the NF1 gene is likely to occur in this particular region, exon 30 - 32.

C-18

PRENATAL DIAGNOSIS OF X-LINKED ADRENOLEUKODYSTROPHY; A POINT MUTATION AT THE ATP-BINDING REGION OF ALDP GENE. Tadashi MATSUMOTO, Tatsuro KONDOH, Tetsuo MATSUSAKA (Dept. Pediatr.), Hideaki MASUZAKI (Dept. Gynecol. Nagasaki Univ. Sch. Med.), Yasuyuki SUZUKI (Dept. Pediatr. Gifu Univ.) and Naoki HARADA (Kyusyu Med. Sci. Nagasaki)

We performed prenatal diagnosis of three fetuses in a X-linked ALD family. The first fetus was supposed to be affected by linkage analysis using polymorphic markers on Xq and by assay of VLCFA-CoA synthetase activity in cultured amniocytes. By sequencing the cDNA from the fetal liver, a G to A substitution was detected, which was thought to result in Arg to His amino acid conversion at codon 617 in the ATP-binding region of ALDP gene. As the Arg at codon 617 is highly conserved among the ATP-binding casette transporter superfamily, this mutation might be the cause of ALD/AMN in this family. The second fetus was normal female. The third fetus was affected by RT-PCR analysis following restriction endonuclease digestion on the RNA from chorionic villi.

C-19

PRENATAL DIAGNOSIS OF TWO FAMILIES CARRING FUKUYAMA TYPE CONGENITAL MUSCULAR DYSTROPHY BY POLYMORPHISMS ANALYSIS

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In 1993, Toda et al. mapped the Fukuyama type congenital muscular dystrophy(FCMD) locus to chromosome 9q31-33. Prenatal diagnoses of two FCMD families are reported using genetic polymorphisms analysis with microsatellite markers flanking the FCMD locus.
There was no consanguinity in either family. The second child in family 1 and the third in family 2 have FCMD, with psychomotor retardation from birth, hyper CKemia, cerebral dysgenesis on brain MRI and severe dystrophic changes in skeletal muscles. Prenatal diagnosis was performed for the third pregnancy in family 1 and the fourth in family 2. Amniocentesis was done at 17w gestational age in family 1 and at 16w in family 2. DNA was extracted from lymphocyte in family members and amniotic cells in fetus. Individuals were genotyped with flanking CA repeat markers and both fetus were analyzed for risk calculation using the LINKAGE package of computor program. The fetus of family 1 had a 99% probability of being a carrier and the fetus of family 2 had a 86% probability of FCMD. The pregnancy in family 1 proceeded and resulted in a healthy baby. Both parents in family 2 decided to have an artificial abortion at 20w gestation. The fetus had vesicular nodules on the cerebral surface, suggesting polymicrogyria in FCMD. A detailed neuropathological study is now underway.

C-20

PITFALL ON PRENATAL DIAGNOSIS OF CONGENITAL LACTIC ACIDEMIA.

Etsuo NAITO, Michinori ITO, Ichiro YOKOTA, Junko MATSUDA, Yasuhiro KURODA (Department of Pediatrics, School of Medicine, University of Tokushima, Tokushima)

In 41 of 260 cultured cells from patients with congenital lactic acidemia, we were able to define the defects. In the prenatal diagnosis of five cases, deficient pyruvate dehydrogenase complex (PDHC) activity was found in the cultured amniocytes obtained from a pregnant woman at risk for PDHC deficiency. The diagnosis was confirmed by the demonstration that PDHC activity was extremely low in the examined tissues from the aborted fetus. Cultured amniocytes and cultured chorionic villus fibroblasts of four other cases showed normal enzyme activities, and two boys and two girls were born after an uneventful pregnancy. In more than 12 months since the children's birth, no symptoms of lactic acidemia have been observed. However, enzymatic examination for the prenatal diagnosis of congenital lactic acidemia deserves particular attention in cases of female E1α deficiency and disorders of mitochondrial DNA, since these defects differ from cell type to cell type in one patient, which can cause tissue-specific deficiencies of these enzymes.

C-21

IDENTIFICATION OF Gly862-> Ser SUBSTITUTION IN TYPE I COLLAGEN GENE (COL1A1) FROM SINGLE SPERM CELLS : A PRACTICAL MODEL FOR PREIMPLANTATION DIAGNOSIS.
Tadashi IIDA, Mieko MATSUSHIMA, Yuka HAYASHI, Mitsuyo TANEMURA, Manami KAWAMURA, Setsuo OKADA, Kaoru SUZUMORI, Yoshiaki YAGAMI (Dept. Obstet. Gynecol., Nagoya City Univ. Med. Sch., Nagoya) and Akira HATA (Dept. Public Health, Hokkaido Univ., Sapporo)

Single sperm cells from father of two patients with type III osteogenesis imperfecta were separated by dilution and micromanupulator technique. A gene segment of type 1 collagen gene containing the affected region was amplified by nested PCR and was sequenced. Among forty individual sperm cells fifteen specimen were identified as a mutant with substitution of G 3208 to A (Gly862 to Ser) while eighteen and seven specimen showed wild type and mixed type, respectively. Through this study we have established a molecular procedure that is considered to be a prerequisite for preimplantation diagnosis of genetic disorders with single point mutation.

C-22

CARRIER DETECTION IN A FAMILY WITH THE FRAGILE X SYNDROME.
Ryozo KASAI (Asahigawa Jidoin Child. Hosp., Okayama), Kouji NARAHARA, Kazushiro TSUJI, Yuji YOKOYAMA, Yoshiki SEINO (Dept. Pediatr., Okayama Univ., Okayama), Masatake YAMAUCHI and Tada-aki HORI (Div. Genet, Natl.Inst.Rad.Sci, Chiba).

Cytogenetic and molecular studies were performed for carrier detection on 12 individuals from a family of 12 individuals with the fragile X [fra (X)] mutation. Cytogenetic studies using fluorodeoxyuridine induction showed positive fra(X) in five family members (two brothers, their mother, younger sister and maternal aunt). The remaining seven relatives had normal chromosomes. Southern blot analyses of EcoR I plus Eag I and Pst I digested genomic DNAs revealed that the mother had a premutation and the remaining four fra(X) positive relatives had full mutations. In addition, two out of seven cytogenetically normal relatives (grandmother and maternal uncle) were found to be premutaion carriers. The size of CGG repeats of females in the second generation was more expanded than the male carrier. We conclud that molecular study is neccessary for carrier detection of fra(X) mutation.

C-23

USE OF FISH ANALYSIS ON UNCULTURED AMNIOCYTES IN DIAGNOSIS OF TRISOMY 18 AND IT'S MOSAICS.
Tsunenori MATSUBARA, Yuji YOKOYAMA, Kouji NARAHARA, Shinsuke NINOMIYA, Tomoko ITO, Yoshiki SEINO (Dept. Pediatr., Okayama University Medical School, Okayama)

We tested efficiency of FISH analysis on uncultured amniocytes in the diagnosis of trisomy 18 and it's mosaics, using D18Z1 probe. Uncultured amniocytes from 4 normal fetuses had 2 signals in 83.5 to 93.0% and 3 signals in 1.0 to 6.0% of interphase nuclei, whereas those of a trisomy 18 fetus had 2 and 3 signals in 23.3 and 48% of nuclei, respectively. Artificial "trisomy 18 mosaic" series of 0, 12.5, 25, 50 and 100%, prepared by mixing cultured amniocytes from the normal and trisomy 18 fetuses, showed 2 signals in 91.0, 83.0, 78.0, 65.0 and 16.5% of nuclei, and 3 signals in 1.0, 9.5, 12.0, 30.5 and 77.5% of nuclei, respectively. The difference in signal distribution between the normal and mosaic series seemed to be insufficient to confirm the existence of low level trisomy 18 mosaics. We conclude that FISH analysis of uncultured amniocytes is useful in the rapid prenatal diagnosis of trisomy 18 as an adjunct to karyotyping, but is of limited value in the diagnosis of mosaics.

C-24

MOSAICISM IN AMNIOTIC FLUID CELL KARYOTYPING WHICH WAS SUGGESTED TO BE EPIGNATHUS ORIGIN.
Kumiko IGARASHI , Satoshi ONOGI, Tomohiko ISHIDA, Makoto OHWADA ,Chikara ENDO, Akira SATO (Dept. Obstet. and Gynecol. , Fukushima Medical Collage)

We report a case of Epignathus demonstrated an unusual mosaic karyotype in cytogenetic analysis of amniocyte. The mother was a G5P3 35 year -old woman. She transferred at 26 weeks gestation with the chief complaint of polyhydramnios and fetal neck tumor. Sonographic findings were a solid tumor arising from the mouth with cystic component. The chromosomal analysis by amniotic fluid cells demonstrated three distinct cell lines: [46,XX], [46,XX,-7,+t(1q;7p)] and [47,XX,+t(1q;7p)]. By aspiration biopsy of the tumor, an immature teratoma was strongly suspected.Because of the excessive rate of growth of the tumor, cesarean section was performed at 33 weeks gestation. A 1890g, female infant was delivered.The baby died of circulatory failure during operation of tumor resection. The tumor was diagnosed as an immature teratoma pathologically. Cytogenetic analysis of umbilical cord blood and tumor tissue obtained postnatally confirmed that the abnormality of chromosome #1 observed in amniotic fluid cells was confined to the tumor. To our knowledge, this is the third Epignathus in which a rearrangement involving the long arm of chromosome #1 has been detected.These data suggest that the duplication of 1q may be responsible for the formation of the teratoma.

C-25

KARYOTYPE ANALYSIS OF COUPLES WITH HABITUAL ABORTIONS IN DETERMINING THEIR CAUSES.
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The choromosome aberration is the most frequently attributed cause for an adventitious abortion. On the other hand, available reports suggest the possession rate of abortion factor among women with recurrent abortions at 43.6% and among those with habitual abortions at 82.2%. These high rates, however, can not be fully explained by chromosome aberration alone. In recent years, various examinations have been made into habitual abortions to determine their causes, and, as the result and as can be seen immunologically, the significant progress have been made in investigating the cause and treatment for infertility. The most recommendable practice at the moment is to provide the couples with genetic counseling.

we have made systematic infertility examinations into 140 couples with recurrent and habitual abortions. In the karyotype analyses, the chromosome aberration in either of them was observed in seven couples or 5% of total (4 reciprocal translocations, 2 mosaicisms and 1 inversion). Of the seven cases, four delivered the normal newborns.

We will address the current genetic counseling issues being faced at the gynecological department.

C-26

" PARENTS' RIGHT TO KNOW" IN THE PRENATAL DIAGNOSIS.
Mitsushiro KIDA(Dept.Pedi.Teikyo Univ.School of Med.,Tokyo)

Recently, the doctor–patient relationship has been reassessed from the standpoint of life ethics. In paticular, the issue of the " parents' right to know" in prenatal diagnosis has been drawing attention.

In actual practice, however, fetal chromosome aberrations are detected by prenatal diag–nosis in 5% or less of fetuses examined. Instead, it is generally believed that " pregnant woman concerned about potiential fetal abnormality tend to choose abortion as a result of being deprived of the opportunity for prenatal testing." This belief is supporting by 1989 statistics on artificial abortion performed in Japan in accor–dance with the Eugenic Protection Act, with over 460,000 cases reported, an amount equal to 38.2% of the total number of births. Of the fetuses thus aborted, some 99.9%were aborted on grounds of " mother's health" . In addition, the number of children per marrid couple(total fetilyty rate) as of 1993 dropped below 1.50. In prenatal diagnosis, the rule is not to inform the parents of the sex of the fetus, since this information could be used to decide to termina te the pregnancy. With recent progress in testing techniques, there has been growing abili ty to detect chromosomal aberrations which manifest " clinically normal" traits or to ob–tain DNA findings. To cope with these issues, we suggest the following measures: 1)Propa gate prenatal testing in order to protect the parents'right to know; 2)Establish standards for detarming which test results will be provided to parents and which will not; in partic ular, resolve not to make public to anrelated parsons any test resuts that may be disadvan tageous to the patient; 3)Confirm the parents' desire for prenatal testing before testing, and obtain their written consent.

C-27

ACTUAL SITUATION AND EXPECTATIONS OF GENETIC COUNSELING FOR PATIENTS WITH GENETIC DISEASES AND THEIR FAMILIES. Tomoko HASEGAWA, Keiko IWASAKI(Div. Clin. Genet., Shizuoka Children's Hospital, Shizuoka)
We mailed a questionnaire to patients with genetic diseases and their families to obtain their opinions about genetic counseling. We focused on patients with short limbs in Japan. A total of 178 replies were received, of which 94% were from patients with achondroplasia and hypochondroplasia. Genetic information was received from clinicians in 54%(96), especially pediatricians (50) and orthopedists (20). Mostly counseling was not performed, and referred to clinical geneticists was rare (8). Before 1979, most genetic information given was incorrect. Although from 1980 almost all the information was correct, there was an increased feeling of dissatisfaction from the clients after receiving the information. The reason for this response may have been a deficiency of comprehensive medical care, and a lack of supportive counseling after receiving the genetic information. Although most medical care in Japan is funded by insurance from private companies and the Government, genetic couseling does not yet qualify for insurance legally, and therefore such counseling is not accepted within the Japanese medical system. However, as the genes responding for various diseases are being discovered rapidly, genetic counseling as part of comprehensive medicine will become more and more important for the support of patients who suffer from genetic diseases and their families.

C-28

Genetic Counseling: An opinion survey of members of the Japanese Organization of Rare Disorders (Asebi Society). Yoshimitsu FUKUSHIMA, Hirofumi OHASHI (Saitama Children's Medical Center) and Emiko SATO (Japanese Organization of Rare Disorders, Asebi Society)

Introduction: An opinion survey of clients is very important to establish the genetic counseling system. The conditions of members of the Japanese Organization of Rare Disorders (Asebi Society) are mostly related to genetic disorders. **Methods:** We sent questionnaires to 785 members and received answers from 455 members, which consisted of 198 with neurofibromatosis, 125 with retinitis pigmentosa, 31 with epidermolysis bullosa, 17 with tuberous sclerosis, 7 with Sturge-Weber syndrome, 6 with de Lange syndrome, 4 with Ehlers-Danlos syndrome and 67 with 38 other disorders. **Results:** 1) 91 % of the members cared about the genetic problem of the disorder; 2) 72% had already asked somebody about the genetic problem; 3) 54% had not understood the genetic problem; 4) 24% knew genetic counseling as medical service; 74% did not know it; 5) 25% had already had counseling; 47% wanted to have it; 29% did not want to have it; 6) 83% thought genetic counseling should be offered at hospital as medical care, not at health centers as health service; 7) 62% accepted new technology including carrier detection, presymptomatic diagnosis and prenatal diagnosis if it was available; 7.8% refused it. **Discussion:** Most of the members wanted to have genetic counseling. However, information concerning genetic counseling is not enough; what is genetic counseling or where is genetic counseling available. The system of genetic counseling in Japan should be established for clients who should have easy access to it.

C-29

PRESENT STATUS AND PROBLEMS IN CLINICAL CYTOGENETICS TEACHING IN SCHOOLS FOR MEDICAL TECHNOLOGISTS:BASED ON QUESTIONNAIRE TO THE 79 SCHOOLS IN JAPAN.Nobuo INOUE(Clin.Lab.,Kanagawa Cardiovascular Respiratory Center, Yokohama)and Tetsuroh OKANO(Kitasato Laboratory of Hygenic Sciences,Tokyo)

Regarding the clinical cytogenetics and chromosomal analytical tech niques,in order to grasp the actual status of their inclusion in the curriculum in Japan,an inquiry was made subjecting nationwide 79 training schools for medical technologists.The major items of the inquiry were:1)The presence or absence of an independent systematic special lecture and practical trainig,2)When there was no special course, the presence or absence of the lecture in the related course, 3)The problems for the establishment of the curriculum,and 4)Comment on the start of the licensing system by the Japan Society of Human Genetics related to the chromosomal analysis. As the results of the inquiry,regarding the item 1),the lecture is given in 50% and the practical training is made in 37%,and,regarding the item 2),the lecture is given in 47% oh the total number of schools Regarding the item 3), 43% answer ed impossible because of the limited time for the curriculum,30% pointed out the difficulty of allocationg an appropriate teacher,and 23% complained of the diffculty of getting teaching materials and specimens.With regard to the item 4),generally,an attiude for positive acceptance was observed and 26% expressed an intention to incorporate a basic training in the pregraduation course which can cope with the licensing system.

C-30

POPULATION GENETIC STUDY OF TYPICAL RETINITIS PIGMENTOSA IN JAPAN. Mutsuko HAYAKAWA, Keiko FUJIKI, Kazushige SADO, Atsushi KANAI (Dept. of Ophthalmol., Juntendo Univ., Tokyo), Miyo MATSUMURA and Yoshihito HONDA (Dept. of Ophthalmol., Kyoto Univ., Kyoto), Norio OHBA (Dept. of Ophthalmol., Kagoshima Univ., Kagoshima), Mizuo MATSUI (Dept. of Ophthalmol., Nihon Univ., Tokyo)

A nationwide, multicenter study of typical retinitis pigmentosa was carried out in collaboration with 18 hospitals throughout Japan, for obtaining a recent information useful for a genetic counselling. A total of 534 patients with RP were registered during a six-month period in 1990. We analyzed a genetic heterogeneity of RP based on the parental consanguinity of 434 probands with full family information, 205 males and 229 females with a wide range of ages (mean 46 years). A gradual decline was found in the overall frequency of consanguineous marriage among the normal parents of RP patients. The relative prevalences of inheritance patterns were estimated as ; autosomal recessive : 25.2%, autosomal dominant : 16.9%, X-linked : 1.6% and simplex cases : 56.3%. A comparison of these results with previous studies in Japan revealed a decline in the relative prevalence of autosomal recessive cases and an increase in simplex cases.

C-31

THE INCIDENCES OF NATURALLY OCCURRING BIRTH DEFECTS IN THE NEONATES BASED ON THE DATA OBTAINED IN THE KANAGAWA BIRTH DEFECTS MONITORING PROGRAM (KAMP).

Yoshikazu KUROKI, Kiyoshi IMAIZUMI, Hiroshi KONISHI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

In Kanagawa prefecture, Kanagawa Birth Defects Monitoring Program (KAMP) has been in operation from October of 1981 as the first population-based monitoring system in Japan. It covers one half of the total births in the prefecture, that is 40,000 births annually. All live births and stillbirths are screened for 44 marker malformations by general obstetricians or rarely by general pediatricians within 7 days after birth. The total number of births covered in KAMP during 1981-1993 reached 500,000. Therefore, we calculated the baseline prevalence of each malformation. These baseline data are under-estimated because of the limitations of our methodology. Thus the ascertain-ment ratio for each marker malformation was estimated by the record linkage study between KAMP data and the clinical data registered in Kanagawa Children's Medical Center. Expected incidences of 16 selected malformations, adjusted by each ascertainment ratio, are presented. Some of the incidence per 10,000 births are, anencephaly 4.8, total cleft lip 14.9, anorectal anomaly 5.6, Down syndrome 8.3. These figures can be used as the standard background data in Japan.

C-32

MORTALITY FROM PARKINSON'S DISEASE IN JAPAN, 1950-1992.

Yoko IMAIZUMI and Ryuichi KANEKO (Institute of Population Problems, Ministry of Health and Welfare, Tokyo)

The death rate from Parkinson's disease (PD) were analyzed using Japanese Vital Statistics for 1950-1992. The age-adjusted PD death rate increased statistically significantly with the years between 1950 and 1992 for both sexes. However, declines of the PD death rate were observed among the population under 65 years old, while striking increases were seen among those over 70 years old. The changing patterns in the PD death rate might be explicable by a constantly improving ascertainment of PD, a true rise in the incidence of PD, particularly among the elderly, and a constantly increasing number of elderly people. There were remarkable differences in the PD death rates among the four marital categories in each sex. The mean age at death in PD increased from about 60 years in 1950 to 77 years in 1992 for both sexes.

C-33

ANALYSIS OF BIRTHWEIGHT IN PAIRED TWINS BY USE OF BIRTH CERTIFICATES IN JAPAN. Noriko KATO (The Institute of Public Health, Tokyo) and Akio ASAKA (Yamanashi Medical University, Yamanashi)

In order to clarify percent deviation of birthweight of twins in Japan, 75,434 data of birth certificates form 1988 to 1991 were analyzed.

The pairs of twins were identified by date and place of birth, ages of parents, and gestational age. 32,232 livebirth-livebirth pairs, 679 livebirth-stillbirth pairs, 278 stillbirth-livebirth pairs, and 2,796 stillbirth-stillbirth pairs were identified. Means of percent deviation were 6.41 for livebirth-livebirth pairs, and 8.92 for stillbirth-stillbirth pairs, which were relatively small, and 34.7 for livebirth-stillbirth pairs, and 25.0 for stillbirth-livebirth pairs, which were relatively larger. When percent deviation was larger, proportion of stillbirths got larger and that of livebirths got smaller, such tendency was stronger in like-sexed pairs rather than unlike-sexed pairs. Pairs with twins of unknown sex were with larger percent deviation, although those of stillbirth-stillbirth pairs gestational age smaller than 22 weeks were not so small.

C-34

ANALYSIS OF BIRTH WEIGHT AMONG MULTIPLE BIRTHS IN JAPAN. Akio ASAKA(Dept. Health Sciences, Yamanashi Medical Univ., Yamanashi) and Noriko KATO(National Institute of Pub. Health, Tokyo)

Birth weight of total 75434 individuals was analyzed during 1988-1991 in Japan. They consist of 72869 twins, 2242 triplets, 256 quadruplets, 55 quintuplets and 12 sextuplets(male 37606; female 36265; sex unknown 1563)(live birth 68136; stillbirth 7298). Mean birth weight in male live births was as follows; 2421g(n=33000) in twins, 1801g(n=922) in triplets, 1306g(n=109) in quadruplets, 1088g(n=8) in quintuplets and 630g(n=5) in sextuplets, respectively. The mean birth weight among female live births was, in the above order, 2353g(n=32985), 1724g(n=978), 1259g(n=97), 921g(n=14)(no sextuplet). The weight among male stillbirth, in the same order, 542g(n=3336), 373g(n=151), 257g(n=29), 250g(n=15), 250g(n=2). The weight among female stillbirth, 735g(n=2049), 509g(n=108), 341g(n=11), 264g(n=7), 300g(n=2). The weight among sex unknown still births, 131g(n=1311), 138g(n=74), 130g(n=10), 177g(n=11), 183g(n=3). Birth weight was higher in live births than in stillbirth in each category. In live births, the weight was gradually lower in the order from twins to sextuplets. The weight was higher in males than in females in each category. In stillbirths, the sex difference of the weight was not clear between live births and stillbirths, except sex unknown groups.

C-35

MONOZYGOTIC TWINS OF DIFFERENT APPARENT SEX

Yukifumi YOKOTA, Atsushi AKANE, Nobuyuki FUJINO, Yoshiaki SATO, Akira MATSUNOBU, Nobuo MATSUURA, Tohru MAEDA, Mamoru TADOKORO, Yutaka NAKAHORI and Yasuo NAKAGOME ; Dept. Pediatr.(Y.Y.,N.F.), Obstet. Gynecol.(Y.S.,A.M.), Pathol.(M.T.), Sagamihara Kyodo Hospital, Sagamihara, Dept. Legal Med.(A.A), Kansai Medical College, Ohsaka, Dept. Pediatr., School of Med.(N.M.), School of Nursing(T.M.), Kitasato Univ., Sagamihara, and Dept. Hum. Genet., School of International Health, Univ. of Tokyo(Y.N.,Y.N.), Tokyo, Japan

We report on twins of unlike sex who shared a 45,X/46,X,+mar karyotype. The mar chromosome was found to be Yq$^-$ by DNA analysis. Marker studies, including 8 VNTR loci, yielded a probability of monozygosity of 0.99999996.

C-36

ABNORMAL MYELINATION IN MAGANETIC RESONANCE IMAGING OF THE BRAIN WITH R(18) SYNDROME.

Yae SHIMAKURA[1], Nobuaki IWASAKI[1], Hisako YANAGI[2], Natsuki IMOTO[1], Hironori IMAI[1], Kimiko KOBAYASHI[2], Chieko NAKAHARA[1], Kenzo HAMANO[1], Tadao ARINAMI[2], Hideo HAMAGUCHI[2], Hitoshi TAKITA[1], ([1]Dept. Pediatr. [2]Dept. Med. Genet., Univ. TSUKUBA, Ibaraki)

The proband was a male born in 1991 at 37 weeks of gestation to a 27 years-old G1P1 mother and 29 years-old father, both of whom were healthy and non-consanguineous Japanese. Pregnancy was complicated by oligohydramnions and IUGR from the 20th week of gestation. He was refereed for genetic evaluation, because he had dysmorphic features. At birth, he weighed 2,496 g and was 48 cm long and his head circumference was 47.5 cm.

His abnormalities at birth included left cleft lip & palate, depressed nasal bridge, low posterioe hairline, micrognatia, rocker bottom feet. Echocardiography showed a ASD and ultrasonography detected bilateral hypoplastic kidney. The delayed myelination was found by brain MRI at two years of age. Cytogenetic analysis using high-resolution banding demonstrated 46, XY, r(18)(p11.2 q21.33). Both of his parents's karyotype were normal.

The myelin basic protein gene (MBP) has been mapped to 18q22-q23. Our patient had a deletion at 18q21.33→qter. He had the dysmorphic facial features for the 18q- and abnormal brain myelination. The data suggest that the presence of only one copy of the MBP gene is responsible for delayed myelination.

C-37

A DUPLICATION OF 4q FROM A PARACENTRIC INVERSION. Mihoko NAKAMURA, Yoshio MAKITA,Mitsuo MASUNO,Kiyoshi IMAIZUMI,Yoshikazu KUROKI(Division of Medical Genetics.Kanagawa Children's Medical Center, Yokohama)

We report on a 9-month-old boy with multiple malformation including macrocephaly,large fontanale, frontalbossing ,blephalophimosis, overelapping finger , ulnar debiation, ventricular septal defect,partial iris coloboma , colpocephaly :enlarged occipital horns of the lateral ventricles ,and sever developmental reterdation .His chromosomal analysis showed a recombinant chromosome resulting from maternal paracentric inversion .Duplication of q22 was detected by whale chromosome painting, then his karyotyoe was 46,XY,rec(4)dup(q22)inv(4)(q21.1q31.3)mat. Mother was phenotypicaly normal.

The mechanism of duplication resulting from parental paracentric inversion was reported by Kasai et al.(1985),and they ssuggested such a rearrengement requires unequal crossing-over at the base of the inversion loop . Our patient strongly suport this hypothesis.

C-38

Inverted duplication of chromosome 16 resulted from paternal paracentric inversion. Kiyoshi IMAIZUMI, Mihoko NAKANURA, Yoshio MAKITA, Mitsuo MASUNO, Yoshikazu KUROKI(Div. Med. Genet.,Kanagawa Child. Med. Cent.)

Chromosomally umbalanced offspring resulting from the recombination of parental paracentric inversion has been considered to be rare. We present a case of inverted tandem duplication resulting from paternal paracetnric inversion. The patient was the third products of a healthy 36-year-old gravida 5, para 3,ab 2 mother and an unrelated 36-year-old father. He was born at 40 weeks of gestation and birth weight was 2280g. He showed short statue, microcephalus, peculiar facies and severe mental retardation. Chromosomal examination of the proposita was 46,XY,inv dup(16)(q22q23) and his father showed 46,XY,inv(16)(q22q23). Chromosomal duplication was proved by fluorescence in situ hybridization(FISH) using whole chromosome painting probe. Various mechanisms have been proposed to explain the structure of the recombinant chromosome and we speculated that U-type re-union within the loop was most probable in our case. It was strongly suggested that de novo duplication and/or deletion might be resulted from parental undetected small paracentric inversion and a tiny duplication or deletion might be found in the malformed inversion carrier offspring of phenotypically normal parents with identical chromosome rearrangements.

C-39

A PATIENT WITH 6q MONOSOMY AND 7q TRISOMY DERIVED FROM PATERNAL TRANSLOCATION.

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The patient, a female neonate, was born at 32 weeks gestation with birth weight 2370g. She had following clinical features: frontal bossing, wide frontal suture, hypertelorism, strabismus, small palpebral fissures, low-set ears, flat nose, cleft palate, micrognathia, short neck, spina bifida, scoliosis, dislocation of the hip, club foot, overlapping toe. In addition to the above malformations, brain CT and MRI showed congenital hydrocephly, hypoplasia of corpus callosum, and type 2 Chiari malformation. Chromosome analysis revealed a structual aberration of the long arm of chromosome 6 in the patient. Her father had a balanced reciprocal translocation between 6q27 and 7q32. The patient had the derivative chromosome 6 and was affected with both 6q distal patial monosomy and 7q distal patial trisomy. This is the first report of the case complicated congenital hydrocephaly in 7q trisomy syndrome.

C-40

FAMILIAL TRANSMISSION OF RECIPROCALLY TRANSLOCATED CHROMOSOMES (2).

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Familial reciprocal translocations ascertained for birth of malformed children were collected from our records and published papers. Of 228 cases with balanced conditions for two or more generations, 113 male and 115 female probands were counted, while their balanced fathers and mothers were 70 and 158, respectively. Furthermore, male and female carriers ascertained through the probands were 82 and 124 of grandparents and also 15 and 23 of great-grandparents, respectively. On the other hand, delivery records of balanced carriers in sibs of parents and grandparents and cousins of parents were examined, and there were no difference by sex among their children with balanced or unbalanced chromosome constitutions. However, males in 54.1% and females in 75.5% of the balanced carriers have experienced childbirth, and this phenomenon was corresponded to sex ratio of grandparents and great-grandparents. These differences of firtility and children's number between male and female carriers must be directly reflected to the excess existence of balanced mothers in data obtained through unbalanced probands.

C-41

A FETUS WITH TWO INDEPENDENT TRISOMIES 13 AND 18: MECHANISM OF THE MOSAIC DEVELOPMENT

Kyohko ABE, Naoki HARADA (Kyushu Med. Sci., Nagasaki), Osamu HIRAKAWA, Junko KIMOTO (Dept. Gynecol. Obstet., Tokuyama Chuo Hosp., Tokuyama), Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Chromosome analysis of amniotic fluid cells from a 32-week-old fetus suffering from polyhydramnios revealed mixoploidy composed of full trisomies 13 and 18. Each trisomy was observed in different cells of different tissue of the fetus. We studied the origin and the mechanism of formation of this unique mixoploidy by tracing chromosomal heteromorphisms as genetic markers. All the heteromorphic markers examined showed no discordance in parent-child transmission or between the two cell lines. The result indicated that the mixoploidy is not chimerism but is mosaicism. Thus it is most likely that two non-disjunctions occurred simultaneously at 4-cell stage of a zygote leading to the two independent trisomies, followed by cell death of consequent two monosomic cells. The baby was born at 37 weeks with a birth weight of 2,680 g and found to have microcephaly, hypertelorism with strabismus, low-set and malformed ears, cleft lip and palate, a congenital heart disease, and bilateral simian creases. Although these clinical features are those mainly for trisomy 13, karyotyping of the cord blood and chorionic villi performed at birth showed only trisomy 18.

C-42

A CASE OF FAMILIAL PERICENTRIC INVERSION OF CHROMOSOME 11 DETECTED PRENATALLY.

Katsuhide ENDO, Takashi YANAGIDA (Dept. Obstet. Gynecol., Seibo Hospital, Tokyo)

A pericentric inversion of chromosome 11 was detected by amniocentesis on a 29-year-old gravida 0 Jewish woman who wished to have a prenatal screening for Tay-Sachs disease. The karyotype was 46,XX,inv(11)(p13q23.3). Genetic investigation on parents revealed the same inverted chromosome in the apparently normal father whose karyotype was 46,XY,inv(11)(p13q23.3). Precise ultrasound study failed to demonstrate any fetal structural abnormalities. Enzyme(β-Galactosidase, β-Hexosaminidase and β-Glucosidase) activities in amniotic fluid cells revealed normal. At 37 weeks of gestation, a caesarean section was performed because of breech presentation to deliver an apparently normal girl. She was later found having mild phenylalaninemia and needed mild diet therapy. Family members of the father were examined genetically and his sister was also diagnosed to have the inversion of chromosome 11. Since his father had normal karyotype, it was supposed that this inversion originated with his deceased mother.

C-43

OUTCOME OF PRENATAL DIAGNOSIS FOR PREGNANCIES OF CHROMOSOMAL TRANSLOCATION CARRIERS IN RELATION TO THE PREGNANCY HISTORY.
Kodo SATO (Dept. Obstet. Gynecol., Toranomon Hospital, Tokyo), Junko IMAMURA and Ryou NAKASONE (Dept. Hematol., Toranomon Hospital, Tokyo)

The frequency of fetuses with unbalanced chromosome constitutions in prenatal diagnosis for pregnancies of chromosomal translocation carriers were discussed in relation to the outcome of previous pregnancies. Seventy nine prenatal diagnosis carried out at Toranomon Hospital between 1986 and 1993 by amniocentesis and/or chorionic villi sampling because of parental chromosome translocations were subjected to analysis. Of the 79, 47(Group A) were ascertained through recurrent miscarriage, 22(Group B) were through prior full-term unbalanced progeny, and the other 10(Group C) were through other abnormal family histories. The frequency of fetuses with unbalanced chromosome constitutions were much higher in Group A than in Group B (21.3% versus 0.0%; in Group C, 10.0%). No significant differences were observed in the type of translocation, i.e. reciprocal or Robertsonian and in the parental or maternal carrier among the three groups. Of the total 79, 31 had no previous history of miscarriage, 23 had histories of one miscarriage, 25 had histories of two or more miscarriages. Ten (32.3%) fetuses out of the 31 with no previous history of miscarriage had unbalanced chromosome constitutions, whereas 1 (4.3%) out of the 23 with histories of one miscarriage, and 0 (0.0%) out of the 25 with histories of two or more miscarriages. These results suggested the importance of evaluation of the previous pregnancy outcome in the genetic counselling for those with chromosome translocations.

C-44

OBSERVATION OF HUMAN LYMPHOCYTES AT HIGH PRESSURE.
Takako TAKANO, Yasuko YAMANOUCHI (Dept. Hygiene & Pub. Health, Teikyo Univ. School of Med.) and Kaoru J. TAKANO (Institute of Materials Science, Univ. of Tsukuba)

Studies of viability, cell cycle and metabolism of human cells at high pressure are important for Genetics and Medicine. We report viability and DNA synthesis of human lymphocytes at the pressures up to 400 MPa.

Normal male lymphocytes established by EB cells were used after synchronized cell culture with thymidine. The cells were then pressurelized for 30 minutes at the temperature of 37 ℃ using a high pressure apparatus. Cell count and viability were measured by 0.1% trypan blue dye-exclusion method both before and after compression and for controls without compression. BrdU incorporation to DNA in the S phase was detected by anti-BrdU antibody for the preparation after decompression.

Viability was 100% up to the pressure of 60MPa. Decrease of viability following the sigmoidal curve began to be observed at that pressure. Survival rate was 50% at the pressures of 130-140 MPa. All the cells were dead at the pressure of 200MPa. BrdU uptake rapidly decreased with increasing pressure without threshold, which means that DNA synthesis is suppressed by the pressure before the cell death.

C-45

THE SECOND CASE OF BLOOM SYNDROME IN A LOCAL AREA.
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Bloom syndrome ia an extremely rare, autosomal recessive disorder, characterized by intrauterin and postnatal growth retardation, photo-sensitive telangiectatic erythema, characteristic face with malar hypoplasia, immunological disorders and a predisposition to malignant neoplasms at a relatively young age.
The patient was a girl at 17 months of age, referred because of growth retardation. She was 6275g in weight and 66.5cm in height. She was born at full term, weighing 1785g, and was 45cm in height without catch up growth. She was noticed to have prominent ears, small face, malar hypoplasia, small mandible and small patches of hyper-pigmentations of skin. She had no telangiectatic erythema on the face nor photo-sensitivity. From her characteristic face and growth pattern, chromosome analysis was obtained and revealed a greatly increased level of spontaneous sister chromatid exchange. No Gaps nor breaks were observed. The parents were healthy and unrelated. But the family lived in a local area where the first case of Bloom syndrome was identified in 1980.

C-46

STRUCTURE AND LOCALIZATION OF THE GENE ENCODING HUMAN PERIPHERAL MYELIN PROTEIN 2 (PMP2).
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Peripheral myelin protein 2 (PMP2) is a basic and cytoplasmic lipid binding protein of peripheral myelin. In this study, we describe the cloning, characterization, and chromosomal mapping of the human PMP2 gene. The gene is about 8 kb in length and consisted of 4 exons. All exon-intron junction sequences conform to the GT/AG rule. The 5'-flanking region of the gene has a TA-rich element (TATA like box) and a single defined transcriptional initiation site detected by the primer extension method. The gene for human PMP2 was assigned to 8q21.3-q22.1 by spot hybridization of the flow-sorted human chromosomes and fluorescence *in situ* hybridization. Recently, Othmane et al. mapped the locus of the autosomal recessive type of Charcot-Marie-Tooth disease (CMTAR) to chromosome 8q13-q21. We preliminary studied five families of CMTAR about PMP2 gene using PCR and could not find any mutation in the coding region of PMP2 gene.

C-47

ANALYSIS OF THE MYELIN PROTEOLIPID PROTEIN GENE IN PELIZAEUS-MERZBACHER DISEASE.
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Pelizaeus-Merzbacher disease (PMD) is an X-linked neurological disorder characterized by dysmyelination in the central nervous system (CNS). Recently mutations of the myelin proteolipid protein (PLP) gene which encodes both PLP and its isoform, DM-20 generated by alternative splicing, have been demonstrated in PMD patients. We analyzed the seven exons of the PLP gene of a Japanese boy affected with PMD by direct sequencing and identified an insertion event in exon VII of the PLP gene. This mutation was also present in his carrier mother, but was absent in ninety-five X chromosomes of normal Japanese. The frame-shift mutation leads to the production of truncated PLP with altered carboxyl terminal amino acid sequences, resulting in considerable change of the structure of PLP and DM-20 necessary for functional purposes. This is the first report of a mutation in exon VII of the PLP gene associated with PMD.

C-48

SPORADIC ALZHEIMER'S DISEASE AND POLYMORPHISM OF *C-FOS* GENE.
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Alzheimer's disease (AD) is a brain disorder characterized by a progressive dementia in the elderly. The pathologic characteristics are cerebral cortical amyloid plaques, neurofibrillary tangles, and amyloid deposits within the walls of leptomeningeal vessels. The major component of the amyloid fibrils in the brains of patients with AD is amyloid precursor protein, APP. The role of genetics in the etiology of Alzheimer's disease has been studied. Recently, several studies have revealed that a major gene for early-onset familial AD has been located to 14q24.3-q31. The *c-fos* gene is a candidate gene for AD since it could be involved in the regulation of APP expression. Here, we have analysed an association between sporadic Alzheimer's disease and *c-fos* gene polymorphisms existed in exon 2 and intron 2. We have examined 70 unrelated Japanese patients with NINCDS-ADRDA sporadic disease and 69 normal controls to determine the frequency of those alleles. No significant difference in frequency of the alleles was found between case and controls.

C-49

APOLIPOPROTEIN E5 AND E7 IN SENILE DEMENTIA OF ALZHEIMER TYPE.

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Apolipoprotein E (apoE) is a polymorphic protein present in plasma lipoproteins and brains. There are three major alleles ε 2, ε 3, ε 4 and two minor alleles ε 5, ε 7 designated according to the reverse order of mobility of isoelectric focusing method. It is estimated that 2 to 3 % of Japanese have either apoE5 or apoE7. Recently, it has been reported that ε 4 allele is a strong risk factor for Alzheimer's disease (AD) and that ε 2 allele shows a protective effect for AD. If the difference of charge of the apoE is associated with the opposite actions of the ε 2 and ε 4 allele, the allele frequencies of ε 5 and ε 7 may also increase in AD. We determined apoE phenotypes of 241 AD patients and compared them with those of 55 age-matched controls (control 1) and 608 apparently healthy Japanese males (control 2). Allele frequencies of apoE in AD patients were ε 2=0.012, ε 3=0.654, ε 4 =0.330, ε 5=0, ε 7=0.004, respectively. Allele frequencies of apoE in control 1 were ε 2=0.045, ε 3=0.845, ε 4=0.082, ε 5=0, ε 7=0.027, respectively. Allele frequencies of apoE in control 2 were ε 2=0.049, ε 3=0.841, ε 4 =0.095, ε 5 =0.005, ε 7=0.009, respectively. The frequency of ε 4 allele in AD patients was significantly higher than those in both controls. In addition, the frequency of E2-positive subjects in AD patients was significantly lower than that in control 2. No associations were observed, however, between apoE5 or apoE7 and AD. Difference of the total charge of apoE may not play important role for AD.

C-49-2

DISTRIBUTION OF APOE GENOTYPES IN JAPANESE CENTENARIANS.

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In order to search for the association between dementia and apo E genotypes in the centenarians, all centenarians (35) living in Yamanashi prefecture in Japan were collected. The blood samples of 33 centenarians were available for extraction of genomic DNA. Ten centenarians were diagnosed as Alzheimer type dementia and one as mixed type dementia in 11 centenarians were demented. We observed the genotypes of apoE using PCR methods. The number of the genotype ε 2/ ε 3 was 3, they all were not demented. Ten out of 27 centenarians genotyped as ε 3/ ε 3 were demented and one out of 3 genotyped as ε 3/ ε 4 were demented. Furthermore, we investigated the distribution of apoE genotypes in a large number patients affected with Alzheimer disease(AD) . The ε 4 allele frequency was significantly increased in AD. Our result was similar to previous reports. The frequencies of alleles were as follows : ε 2 was 2.5%, ε 3 was 70.4% and ε 4 was 27.2% in 162 patients with AD, and ε 2 was 4.1%, ε 3 was 88.2% and ε 4 was 7.7% in 110 controls.

C-50

ANALYSIS OF SEROTONIN 1A RECEPTOR GENE by PCR-SSCP IN BIPOLAR AFFECTIVE DISORDER. Jun SAKAI, Yasuhiro INAYAMA, Hiroshi YONEDA, Toru ISHIDA, Yasuhiro NONOMURA, Yoshihiro KONO, Ryuichi TAKAHATA, Jun KOH, Ryuichiro HIROTA, Yasushi INADA, Toshiaki SAKAI (Dept. of Neuropsychiat., Osaka Medical College , Osaka)

Family, twin and adoption study have shown that genetic factors play an important role in the etiology of affective disorder. Although linkage and association studies using DNA markers have been conducted, their results remain inconclusive.

The serotonin 1A receptor (5HTR1A) gene is thought as a candidate gene for affective disorder by its pharmacological and biochemical studies. The gene is located on chromosome 5q11.2-13, and is intronless with a coding region of 1263 base pairs. We investigated the polymorphism of 5HTR1A gene by the PCR-SSCP method.

The subjects were 40 bipolar disorders and 30 normal controls. We extracted DNA from blood samples and amplified 5HTR1A gene by PCR. 5HTR1A gene was divided into four region. Four couples of primers covering whole coding region were designed. PCR products were denatured, loaded onto a 10% polyacrylamide gel containing 5% glycerol, and electrophoresed at 4℃.

Two bipolars and one control showed different band pattern in a quarter region from the 5' end. However the frequency of different band was not different between bipolars and controls. In the three quarters of the amplified region from the 3' end, we found no different band pattern in bipolars and controls. These results suggest that 5HTR1A gene may not be a main etiological factor of bipolar disorder.

C-51

Polymorphism of interleukin 2 receptor gene in schizophrenia Masahiko TATSUMI[1], Toshiyuki SAKAI[1], Mineko HATTORI[2], Shinichiro NANKO[2], Hajime KAZAMATSURI[2], Kunitoshi KAMIJIMA[1]
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Recently,there have been reported that aberrations of interleukin 2 (IL-2) in cytokine were found in the patients with schizophrenia, for example decrease of the IL-2 production and increase of the soluble IL-2 receptor. Moreover, Pulver et al. (1993) suggested a linkage between schizophrenia and IL-2 receptor B (IL-2RB) gene on chromosome 22. Thus, the IL-2RB gene is one of the candidate gene for schizophrenia. Using dinucleotide repeat polymorphism of the IL-2RB gene, we have carried out an association study between 54 patients with schizophrenia and 54 controls, as well as a linkage study in six multiply affected pedigrees. No significant difference was found between patients and controls. For linkage study, LOD scores were negative for all phenotype definitions as well as for all genetic models. These results indicated that the IL-2RB gene is less likely to be a major facter to cotribute the pathophysiology of schizophrenia.

C-52

AN ASSOCIATION STUDY BETWEEN SCHIZOPHRENIA AND DOPAMINE TRANSPORTER GENE (DAT). Toru ISHIDA, Hiroshi YONEDA, Yoshihiro KONO, Yasuhiro NONOMURA, Yasuhiro INAYAMA, Ryuichi TAKAHATA, Jun KOH, Jun SAKAI, Ryuichiro HIROTA, Yasushi INADA and Toshiaki SAKAI (Dept. Neuropsychiat., Osaka Medical College, Takatsuki)

The hyperactivity of dopaminergic system is one of the major etiological hypotheses of schizophrenia. Giros et al (1992) have cloned the human dopamine transporter gene(DAT) which reuptakes and inactivates dopamine. Vandenbergh et al (1992) have reported a 40-bp variable number tandem repeats (VNTR) ranging from 3 to 11 copies in the 3' untranslated region of this cDNA. We investigated this VNTR in 50 biologically unrelated Japanese schizophrenics and 50 controls. Genomic DNAs were prepared from peripheral white blood cells using standard method. The 40-bp repetitive element in the 3' untranslated region was amplified by PCR. The PCR products were separated by agarose gel electrophoresis, 11(A1:485bp), 10(A2:445bp), 9(A3:405bp), and 7(A4:325bp) copies of the 40-bp repeat were found. The allele frequencies did not show any significant differences between the schizophrenics and the controls. This result suggests that the DAT gene may not directly cause schizophrenia.

C-53

A VARIANT FORM OF DOPAMINE D2 RECEPTOR AND SCHIZOPHRENIA
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The dopamine D2 receptor gene is a candidate gene for schizophrenia because the potency of certain neuroleptics correlates with their affinity for this receptor. A case-control study in 292 schizophrenia and 579 controls on an association of a molecular variant of S311C of the dopamine D2 receptor with psychiatric disorders was conducted. The frequency of individuals with S311C was significantly higher in schizophrenics with the absence of negative symptoms (17.1%, $p<0.00001$), but similar in schizophrenics with the presence of negative symptoms (5.7%, $p=0.46$) compared with the controls (4.1%). S311C was significantly frequently found in familial schizophrenics in one area, but not in other areas. Schizophrenics with S311C were more frequently married and had more children than those without S311C. These data suggest that S311C might be one of the genes affecting symptomatic dimensions of delusions and hallucinations and be involved in underlying clinical heterogeneity in schizophrenia.

C-54

ASSOCIATION BETWEEN DOPAMINE D4 RECEPTOR GENE AND SCHIZOPHRENIA. Toshihisa TANAKA[1], Shuichi IGARASHI[2], Osamu ONODERA[2], Hajime TANAKA[2], Kensuke KAMEDA[1], Kuniaki TAKAHASHI[3], Shoji TSUJI[2], Shin IHDA[1] 1) Dept. Psychiat., Niigata University, Niigata 2) Dept. Neurol., Brain Research Institute, Niigata University, Niigata 3) Department of Psychiatry, Sado General Hospital, Niigata

The authors examined if there is any allelic association of dopamine D4 receptor gene with schizophrenia. We analyzed 70 unrelated schizophrenic cases and 76 normal controls to determine the allelic frequencies created by length polymorphism of dopamine D4 receptor gene. All patients and controls were unrelated and from the Japanese population. Ptients were divided into two groups with regard to age at onset, famial loading, severity of symptoms asessed strictly with Manchester scale. There were no statistically significant differences if the distributions of alleles and genotypes were analyzed for the entire group of patients with schizophrenia. While there was a trend ($p<0.1$) towards a greater prevalence of 5-repeat allele to be more prevalent among schizophrenics with severe symptoms, this analysis failed to reach statistical significance. The present data indicated that relationship between schizophrenia and dopamine D4 receptor gene polymorphism is unlikely to be present in Japanese patients.

C-55

IMPRINTING MATURATION OF THE H19 GENE IN HUMAN PLACENTAE. Yoshihiro JINNO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Yuichiro IKEDA, Hideaki MASUZAKI, Tadayuki ISHIMARU and Tohru YAMABE (Dept. Obstetr. Gynecol., Nagasaki Univ. Sch. Med., Nagasaki)

Allele-specific expression of the H19 gene was analyzed in human villi and placentae ranged from 6-week to 40-week gestational age. Among 33 informative cases, monoallelic expression was confirmed in 25, while eight showed biallelic expression. This biallelic expression was only observed in villi with 9-week or younger gestational age and did not seem to correlate with WT1 monoallelic or biallelic expression. Furthermore, it did not affect allele-specific expression of IGF2 nor resulted in reduction of IGF2 expression. These observations indicate that biallelic expression of H19 does not result from loss of imprinting or imprinting polymorphism, rather represents a stage preceeding to establishment of H19 functional imprinting and that establishment of functional imprinting is independent each other.

C-56

2N/3N MIXOPLOID IN A PARTIAL HYDATIDIFORM MOLE IDENTIFIED WITH MOLECULAR ANALYSIS

Yuichiro IKEDA, Hideaki MASUZAKI (Dept. Obstet. Gynecol., Nagasaki Univ. Sch. Med., Nagasaki), Yoshihiro JINNO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

The purpose of our study was to know the mechanism of formation of partial hydatidiform mole by tracing the parent-mole transmission of polymorphic DNA markers. Genomic DNA was extracted from a region with molar changes and a non-molar region of an 18-weeks' placenta diagnosed as partial hydatidiform mole. DNAs were also extracted from peripheral blood leukocytes of the parents. DNA polymorphisms of the 8 loci from 6 chromosomes were detected by polymerase chain reaction (PCR) and Southern blot hybridization. Densitometry was carried out to estimate a density ratio of a paternal to a maternal allele-derived electrophoretic band. The results showed that this mole was a product composed of biparental alleles in all the 8 loci examined. Whatever loci were examined, a band density for the paternal allele was higher than that for the maternal allele. A paternal/maternal-allele ratio was estimated to be about 1.5 in all the 8 loci. These results suggested that this partial hydatidiform mole is a mixoploid consisting of diploid/triploid cells at the region with hydropic changes and a normal diploid constitution at regions with non-hydropic changes. It is most likely that an extra paternal haploid set causes hydropic changes in the partial hydatidiform mole. Chromosomal heteromorphism study may be able to differentiate between mosaicism and chimerism.

C-57

DEVELOPMENTAL GENETIC ANALYSIS OF MUTATIONS MAPPED IN MOUSE T/t COMPLEX. Kuniya Abe[1], Tadashi Kaname[1], Karen Artzt[2], Ken-ichi Yamamura[1] ([1] Inst. of Mol. Emb. Genet., Kumamoto Univ., [2]Univ. of Texas at Austin)

The *T/t* complex of mouse is a large genetic region on proximal half of chromosome 17 that carry a number of loci affecting embryogenesis and sperm functions. We have been analyzing this region, attempting to ultimately isolate genes responsible for these mutations. Among these, we have special interests in mutations such as t^{12}, a preimplantation lethal; t^{w5}, showing defects in embryonic ectoderm formation; *qk* (quaking), a neurological mutant. We have mapped t^{12} and t^{w5} within mouse MHC region, H-2 complex. t^{w5} was found to be very close to the H-2K gene with a possible genetic distance less than 0.1 cM. We have cloned genomic region spanning ~800 kb around the K gene into cosmid contigs and have searched and found more than 20 genes within this region. We are now trying to establish the technique of making YAC-introduced transgenic mice, which will enable us to functionalcharacterization of the genomic region.

C-60

MOSAIC EMBRYOS WITH STRUCTURALLY ABNORMAL CHROMOSOMES OCCURRED AT EARLY CLEAVAGE STAGES.

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Mosaic cases having cells with two or more different chromosome constitutions including structural abnormalities have been often seen in human live births and abortions. It is considered that some of them might occur due to two events including nondisjunction and chromosome rearrangements. In some cases, however, their occurrence may readily be explained by chromatid translocations and selective elimination of the segregants. Using Chinese hamsters, we investigated the incidence of chromatid translocation in embryos induced by X-irradiation (1.0-4.0 Gy) and mytomycin C treatment (0.2-5 mg/kg) at various stages. Chromosomes of embryos at the first and second mitotic division were analyzed from the cross between translocation homozygotes. The frequency of embryos with chromatid translocations in 3 Gy-irradiated group were 0.6-7.2 % and 0.2-5.1% at the first and second mitotic division, which did not so differ from the frequency of multivalents at meiosis. In humans, aneuploid and mosaic cases originating from such events at the first and second mitotic division may represent a considerable proportion.

C-61

MECHANISM OF MUTATION IN THE GERMLINE AT HUMAN MINISATELLITES. Keiji TAMAKI (Dept. of Legal Med., Nagoya Univ. Sch. Med., Nagoya), Annette MACLEOD, Darren G. MONCKTON, David L. NEIL, John A.L. ARMOUR, Alec J. JEFFREYS (Dept. of Genet., University of Leicester, Leicester)

Mutation at the human minisatellites MS32, MS205 and MS31A has been investigated by characterizing mutant alleles in pedigrees and in the case of MS32 by direct analysis of mutant molecules in single sperm. Most mutations at all three loci are polar, involving the preferential gain of a few repeat units at one end of the tandem repeat array. Incoming repeats can be derived from the same allele or the homologous chromosome, though they are frequently rearranged during mutation. Lack of exchange of flanking markers suggests the involvement of complex conversion-like events in the generation of mutant alleles. At MS32, high frequency mutation processes in sperm appear to be largely germline specific and to occur at a constant rate irrespective of allele size. Some alleles at minisatellite MS32 show reduced variability in human populations and are associated with a G to C transversion upstream of the array. Analysis of single sperm demonstrated a frequently profound reduction in mutation rate at alleles carrying the C variant. This mutation suppression acts in *cis*, but does not affect the ability of an allele to act as a sequence donor during gene conversion. This mutation rate polymorphism provides strong evidence that elements near the tandem repeat array regulate minisatellite mutation.

C-62

ISOLATION AND DETECTION OF MICROSATELLITES. Tetsuro MIKI, Jun NAKURA, Lin YE, Noriaki MITSUDA, Kouzin KAMINO and Toshio OHIGIHARA (Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka)

A microsatellite has been useful to make a genetic map. We have developed new methods to clone microsatellites from a definite region and to detect polymorphic DNA bands easily. To isolate microsatellites we have introduced two methods; (a) screening microsatellites of a plasmid library which was developed by a chromosome microdissection and enzyme amplification method, and (b) screening microsatellite of a plasmid library derived from cosmid clone or YAC clone. We can detect DNA bands by two methods; (1) if the PCR product is about 100 base pairs, we can find as little as a 2 base pair difference in products employing 10 % polyacrylamide gel electrophoresis after ethidium bromide staining and (2) if we label primers by rhodamine, the images are obtained by scanning the gels with a fluorescent image analyzer.

C-63

SINGLE CELL ANALYSIS ON NUCLEATED ERYTHROCYTES. KAZUMI IKAWA, (ISHIKAWA HEALTH SERVICE ASSOCIATION) KAORU YAMAFUJI, (UKITA HOSPITAL, Kanazawa) HARUO TAKABAYASHI, (KANAZAWA MEDICAL UNIVERSITY)

The advent of the polymerase chain reaction (PCR) enables DNA diagnosis to be performed for a single cell.There is no evidence that target cells on the smear slide can be retrieved selectively and individually, and analysed at a single cell level. We have recently developed a new method for the ditection, retrieval and analysis of single nucleated erythrocytes (NRBCs). Peripheral blood granulocytes including NRBCs isolated by a discontinuous density gradient method using percoll. NRBCs were found and retrieved at a single cell level using a micromanipulator under a microscope. And then NRBCs successfully analysed by PCR to determine the presence of Y-specific repeat sequence and β -hexosaminidase A exon 11. This new technique opens up DNA diagnosis of single target cell on the smear slide. Further refinement in methodology is needed in order to apply this new technique to clinical purposes.

C-64

DNA ANALYSIS OF AN ABNORMAL PLASMINOGEN, PLG M^{Osaka}, BY PCR-SSCP AND NON-ISOTOPIC DIRECT CYCLE SEQUENCING.

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Molecular analysis of an abnormal plasminogen, PLG M^{Osaka}, with inactive molecule (Yamaguchi et.al., 1989) was performed. Genomic DNAs were prepared from peripheral blood lymphocytes, which were obtained from two unrelated healthy individuals showing the PLG A-M^{Osaka} (PLG activity :52% of normal, PLG concentration : 9.1 mg/dl) and PLG M5-M^{Osaka} (21% of normal, 8.4mg/dl) phenotypes and normal control individuals showing the PLG A-A phenotype. All the plasminogen exons were amplified by the polymerase chain reaction (PCR) using a set of 19 pairs of primers. Screening for mutations in the plasminogen gene was carried out by single strand conformational polymorphism (SSCP) analysis using silver staining. The same variant SSCP patterns were detected only in exon 17 fragments amplified from both PLG A-M^{Osaka} and PLG M5-M^{Osaka}. Non-RI direct cycle sequencing of these exon 17 fragments revealed a new mutation from GAC to AAC in codon 676 predicting the change from Asp to Asn at residue 676 in the plasminogen, PLG M^{Osaka}, gene. In addition, the exon 15 fragment of PLG M5-M^{Osaka} displaying a variant SSCP pattern was also sequenced. As a result, the PLG M5 gene was found to be the same as the Type I mutation (from GCT to ACT in codon 601) reported by Ichinose. These findings suggest that these mutations cause structural alterations resulting in drastic decreases in plasminogen activity.

C-65

HETEROGENEITY OF SILENT TYPE HUMAN SERUM CHOLINESTERASE GENE. AN ADDITIONAL EXAMPLE CAUSED BY ADENINE INSERTION AT CODON 315 (ACC).

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Cholinesterasemia associated with deficiency of enzyme activity genetically can developed prolonged apnea after administration of the muscle relaxant drug succinylcholine and is one of the pharmacogenetic diseases. Recently, we carried out DNA analysis in a 64 year-old female with a decreased level of butyrylcholineseterase in serum. DNA was isolated from white blood cells and the abnormal region on exon 2 was demonstrated by SSCP analysis of DNA amplified by PCR method. Directsequencing of the amplified DNA revealed a frameshift mutation produced by an extra A insertion at codon 315 (ACC → AACC), resulting in termination codon at 322. The other two siblings in this family were carriers of this mutation (one homozygote, one heterozygote). Immunoelectrophoresis to detect enzyme protein showed an absence of cross-reactive material. We have already found 3 kinds of silent butyrylcholinesterase variant, namely, Silent-1 (codon 365 G→C), Silent-2 (codon 128 A→G) and Silent-3 (codon 400 C→A).

C-66

A KOREAN FAMILY WITH LESCH-NYHAN SYNDROME AND THE PRENATAL DIAGNOSIS. Yasukazu YAMADA[1], Haruko GOTO[1], Yong CHOI[2], Ki Joong KIM[2], Kaoru SUZUMORI[3], Nobuaki OGASAWARA[1] ([1]Dept. Genet., Inst. Developmental Res, Aichi Prefectural Colony, Aichi, [2]Dept. Pediat. Seoul Natl. Univ. Child. Hosp., Seoul, [3]Dept. Obstet. Gynecol., Nagoya City Univ. Med. Sch., Nagoya)

Complete deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8) causes Lesch-Nyhan syndrome. By the analyses of genomic DNA and mRNA using PCR technique coupled with direct sequencing, we identified a mutation on the HPRT gene in a Korean family, and performed familial gene analysis and the prenatal diagnosis.

A 2-bp deletion of GT at nucleotide position 289 and 290 on the exon 3, has been identified by the analysis of genomic DNA from the patient. By utilizing a *Bfa* I restriction site which was created in the mutation as an indicator, family study was carried out and the mother predicted to be a heterozygous Lesch-Nyhan carrier. The amniotic cells of the mother's second conception were resistant for 6-thioguanine. Further, the genetic analysis of the cells revealed the fetal brother inherited the mutant HPRT gene from the mother. The mutation generated two types of abnormal mRNA; one altered mRNA, Type a, resulted in stop codon (TAA) appearing at codon 107 because of frame-shift of a codon from the mutation site (codon 97) and the other, Type b skipping the exons 2 and 3, is predicted to produce the enzyme protein deleted of 97 amino acid residues.

C-67

A NOVEL MUTATION OF THE PAX3 GENE FOUND IN A FAMILY WITH WAARDENBURG SYNDROME TYPE 1

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Waardenburg syndrome type 1 (WS1) is an autosomal dominant disorder characterized by dystopia canthorum, pigmentary disturbances, and sensorineural deafness. WS1 is caused by mutations of *PAX3* located at 2q35. We have analyzed *PAX3* in 17 WS1 patients from 6 families. The paired domain and octapeptide coding sequences of *PAX3* including adjacent splice junctions were divided into 4 parts, each corresponding to each exon, and they were amplified by PCR using primer pairs with exon-flanking sequences. DNA sequence analysis of cloned samples of PCR products revealed one single-base substitution at exon 2 in one family including 9 patients. This single-base substitution is a hitherto undescribed missense mutation, Ile59Phe, resulting from ATC →TTC. The A to T transversion interrupted a *Sau*3AI site, inhibiting enzyme cutting of the mutated allele. The mutation was identified in all nine affected individuals in this family and easily confirmed by *Sau*3AI digestion of PCR products for exon 2. This system is useful for prenatal diagnosis in the family.

C-68

N-acetylation polymorphism in sarcoidosis.

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N-acetylation polymorphism is a representative of pharmacogenetic traits determined by polymorphic N-acetyltransferase activity in the liver. It has been implicated in the susceptibility to some multifactorial diseases.We have investigated the possible association between N-acetylation polymorphism and sarcoidosis.The acetylator genotype was determined by a PCR-based RFLP method as previously reported,using the genomic DNA extracted from leucocytes in 100 patients with sarcoidosis and 329 normal subjects.An additional variant "M4" was screened by another PCR-RFLP.The distribution of presumed acetylator phenotype in sarcoidosis was not significantly different from that in normal subjects.However, the distributions of presumed acetylator phenotype in patients accompanied by skin manifestation and in patients who showed good clinical course during two year treatment were significantly diferent from normal subjects.A rare variant M4 was not detected in the Japanese population.

C-69

MOLECULAR HETEROGENEITY OF PYRUVATE KINASE (PK) DEFICIENCY IDENTIFIED BY SINGLE STRAND CONFOR-MATIONAL POLYMORPHISM (SSCP) ANALYSIS.

Hitoshi KANNO, Shiro MIWA (Okinaka Memorial Institute for Medical Research) and Hisaichi FUJII (Dept. Blood Transfusion Medicine, Tokyo Women's Medical College)

To elucidate the molecular heterogeneity of the PK deficient hemolytic anemia among Japanese population, we analyzed the seventeen Japanese families of PK deficiency by the PCR-SSCP analysis. Nine missense mutations, a three base insertion and a one base deletion were identified. Among the missense mutations six were found to be novel. The 1468T was identified in nine unrelated families, suggesting that the most prevalent mutation among the Japanese PK variants. Although the previous studies revealed that the 1529 A was most dominant PK mutation among the European population, no Japanese PK variant had the mutation. The three bases in-frame insertion of exon 6 (664-6 GAC, 222Asp) was detected in three unrelated families. *PK Beppu,* one of the most severe PK variant, had been shown that the M2-type PK persists in mature red cells. We found that the variant was homozygous with a one base deletion (del 434C) of the L-PK gene, resulting in the frame-shift and premature termination of translation. The truncated R-PK subunit lacks about 2/3 of the C-terminal portion. The affected red cells may survive by a compensatory M2-PK expression. Our present studies showed that among the 38 mutations (28 missense, 1 nonsense and 2 splicing mutations , 3 insertions and 4 deletions), only two missense mutations (1151T and 1436A) were found in both Europeans and Japanese.

C-70

MOLECULAR ANALYSIS OF THE Y CHROMOSOME LONG ARM IN AZOOSPERMIC PATIENTS.

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(Dept. Hum. Genet., Tokyo Univ., Tokyo., [1]Dept. Urol., Osaka Univ., Osaka.)

We analyzed DNA from 110 Japanese men with either azoospermia or severe oligospermia whose Y chromosomes were cytogenetically normal. A total of 16 loci were examined; 15 loci on the long arm between DYS7E and DYZ1, and the YRRM1 locus, a candidate gene for AZF. We detected micro-deletions in 15 of the 110. The YRRM1 gene was involved in only three of them. The remaining 12 patients showed possible deletion between DYS7C and DYS239 in common. These facts indicate the presence of at least an additional gene deletion of which causes azoospermia. The cloning of the gene is now in progress.

C-71

ANALYSIS OF SRY GENE IN 46, XY SEX REVERSAL PATIENTS BY PCR SSCP AND PCR DIRECT SEQUENCING.

Taku IIDA[1,2], Rie KOMAKI[1], Nobuyoshi HAYASHI[3], Yutaka NAKAHORI[1], Yasuo NAKAGOME[1] ([1]Dept. Hum. Genet., Tokyo Univ., Tokyo, [2]Dept. Obstet. and Gynecol., Tokyo Univ., Tokyo, [3]Dept. Gynecology ,Okayama Central Hosp.,Okayama)

We performed the PCR-SSCP and PCR-direct sequencing analyses of SRY in 22 patients who had a 46, XY karyotype and the female external genitalia. Patient with testicular feminization were not includes in the present study.

Two cases lacked the SRY region presumably due to X-Y translocation. The SRY gene of the remaining 20 cases were studied by the single strand conformation polymorphism (SSCP) method. And one of the patients showed an abnormal band pattern in electrophoresis.

Direct sequencing was carried out using a biotinylated and a non-biotinylated primers. A single base substitution was detected in the codon 107.

We also examined the sequence of SRY in the patient's father. As expected, the base substitution in the patient was *de novo*.

The patient was a 28-year-old married Japanese woman with a history of primary amenorrhea and infertility. Physical examination revealed an apparently normal female with an underdeveloped uterus. Normal fallopian tubes were identified in the laparotomy.

C-72

LAPAROSCOPIC SURGERY AND AND DNA ANALYSIS IN XY PURE GONADAL DYSGENESIS. Osamu TSUTSUMI, Taku IIDA, Yutaka MORITA, Yuji TAKETANI (Dept. Obstet. Gynecol., Univ. Tokyo, Tokyo) and Yutaka NAKAHORI, Yasuo NAKAGOME (Dept. Human Genetics, Univ. Tokyo, Tokyo)

The XY pure gonadal dysgenesis is characterized by streak gonads in phenotypic females who lack the somatic abnormalities and short stature associated with Turner's syndrome. Abnormalities within the SRY have been described in these patients. However, we have experienced several patients with short stature whose SRY are apparently normal. The DNA sequencing of the SRY gene showed a 100% nucleotide sequence identity with the reported cloned sequence. Sex reversal in two of the present cases may be due to mutation at a locus other than SRY in the sex determining pathway, a gene potentially involved in the determination of human constitution. The risk of developing malignancy in the dysgenetic gonads has been reported to be 25%, dictating early prophylactic removal of the streaks. Laparoscopic surgery is recommended because of the amount of the surgery and the rapid postoperative recovery of the patient.

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