日本人類遺伝学会 第35回大会一般講演要旨

Abstracts of General Contribution, the 35th Annual Meeting of the Japan Society of Human Genetics

A 1

FAMILY OF MAN

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There are various hypotheses regarding the origin of man but the mystery has not been resolved yet. Perhaps man originated in Central Asia. When man's family size increased, its members migrated in different directions. Mutations, natural selection and genetic drift played their roles in bringing about differentiation.

Hunter and gatherer gradually became an organised and mechanized man. As man's family continued to increase, conflicts arose. Some of the conflicts turned into wars. The climax came when atomic bombs were dropped at Hiroshima and Nagasaki. Devastation was unparalleled. But there was a lesson to learn: If discretion was not used, human species could become extinct. After all, man belongs only to one family. Why destroy it!

A 4

The relationship between the Xmn I site polymorphism 5' to the G γ -globin gene and its gene expression in a Japanese population with or without elevated Hb F.

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It is well known that the G γ /A γ ratio of the newborn is about 7/3, while that of the adult, around 2/3. Its abnormal ratio depends on the γ -globin gene number and the abnormality of its promoter or enhancer sequence. It has been said that the C $\rightarrow$ T substitution at -158 bp to the G γ -globin gene cap site, which produces the presence of the Xmn I site, accompanies the elevation of the G γ -globin chain synthesis. We have succeeded in the enrichment of fetal hemoglobin (Hb F) from the Hb solution of the normal adult with less than 1% HbF. We have analysed the G γ /A γ ratio and the Xmn I site polymorphism 5' to the G γ -globin gene in adult Japanese blood donors with or without elevated Hb F. Our data have shown that the presence of the Xmn I site 5' to the G γ -globin gene should be related to the high level expression of the G γ -globin gene. Interestingly, the presence or absence of the Xmn I site 5' to the G γ -globin gene is always in accordance with the presence or absence of the Hinc II site in the $\psi\beta 1$ -globin gene, respectively. The frequency of the Hinc II site in the $\psi\beta 1$ -globin gene has been known to be different among different ethnic groups. The frequency of the Xmn I site 5' to the G γ -globin gene was 0.17, and consequently the G γ /A γ ratio in a normal Japanese population should be somewhat lower than 2/3.

A 5

HEMOGLOBINOPATHIES/THALASSAEMIA IN NORTH INDIA

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In North India, the more common α globin chain variants include HbJ-Paris I, HbQ-India and β globin chain variants include HbD-Punjab and HbE. We have also discovered rare variants like Unstable Hb, HbG-Norfolk and a new Hb-Chandigarh.

Both α and β thalassaemia are prevalent in north India. The α thalassaemia is α^+ type and is clinically asymptomatic. Only 3 cases of HbH disease has been encountered in the last 15 years. Both β^0 and β^+ thalassaemia is equally distributed in the population. Occasional case of $\delta\beta$ thalassaemia and HPFH is also seen.

The distribution of thalassaemia and hemoglobin variants can be related to environmental factors such as malaria & migration of Punjabi people

A 7

RACIAL DIFFERENCES IN RESTRICTION FRAGMENT LENGTH POLYMORPHISMS.

Atsushi AKANE, Kazuo MATSUBARA, Setsunori TAKAHASHI, Hiroshi SHIONO (Dept. Legal Med., Shimane Medical Univ., Izumo), Shin-ichi YOKOTA, Masao YAMADA and Yasuo NAKAGOME (Dept. Congen. Abnormal. Res., Natl. Child. Med. Res. Cent., Tokyo).

We have analyzed several probes for restriction fragment length polymorphisms (RFLPs), which were established after screening of DNA samples from Caucasian, to check allele frequencies in Japanese population. Then two Japanese-specific RFLPs which were monomorphic in Caucasian and seven Caucasian-specific RFLPs which were almost monomorphic in Japanese were discovered. These RFLPs were also analyzed in three Chinese populations (Guangzhou, Beijing and Shijiazhuang), and compared to those in Japanese and Caucasian. Three monomorphic RFLPs in Japanese were entirely monomorphic in Chinese. Four little-polymorphic RFLPs in Japanese were also little-polymorphic in Chinese, and one of two RFLPs specific to Japanese revealed the similar allele frequencies in Chinese populations.

A 8

Evolutionary pattern of nucleotide substitution in HLA class I molecules : Overdominant selection must occur on ARS and cytoplasmic domains.

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HLA (Human Leucocyte antigen) loci are well known to be highly polymorphic. By comparing 41 nucleotide sequences of HLA class I molecules, we examined the pattern of nucleotide substitution between polymorphic alleles in the regions of the antigen recognition site (ARS), and other regions of human HLA class I genes. As pointed by Hughes and Nei (1988), non-synonymous substitution is higher than that of synonymous substitution in ARS. In addition to ARS, we could find the similar pattern in cytoplasmic domain (CY domain) of class I molecules. This suggests that overdominant selection also operates on this domain. Functional importance of CY domain was compared with ARS and hypervariable regions of IgG. Hughes, A.L., Nei, M. (1988) Nature 335, 167-170.

A 9

COMPLEMENT C4A DUPLICATION ON COMMON MHC HAPLOTYPES

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The most predominant major histocompatibility complex (MHC) haplotype HLA A24-Bw52-C2C-BFS-DR2(w15)-DQw1(w6) was shown to carry a duplication of complement C4A gene. Protein typing on the homozygotes for this haplotype showed two C4A allotypes, A3 and A2, but no C4B allotype. The RFLP analysis using Taq I suggested that one C4A gene is on the 6.0kb fragment and another C4A gene on the 7.0kb fragment. The analysis using pulsed field gel electrophoresis further supported that both C4 loci are occupied by the C4A genes. This haplotype exists at 1-3% in northern China and Korea but is rare in southern China and Taiwan, suggesting a migration route of a Japanese ancestor group from northern China to western Japan. Interestingly, another common MHC haplotype HLA A24-Cw7-B7-C2C-BFS-C4A3+3-C4B1-DR1-DQW1(W5) also carries a C4A duplication, while the deletion of C4A gene is rare in Japanese.

A 10

GENETIC POLYMORPHISM OF APOLIPOPROTEIN E AND HYPERLIPIDEMIA IN JAPANESE: VI. FREQUENCY OF APO E5 & E7 AND SERUM CHOLESTEROL LEVELS.

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Frequencies of apo E5 and E7 were examined in Japanese males under 65-year-old, who visited to kudanzaka Hosp., Tokyo, for their annual health care examinations. The males with history on operation of a digestive system, abnormalities in liver and renal functions, or diabetes mellitus were excluded. ApoE phenotypes were determined in 615 males by two-dimensional gel electrophoresis using VLDL-rich fractions. The frequency of individuals with apo E5 was 0.9 % (four apo E3/5, one apo E2/5). The frequency of individuals with apo E7 was 1.8% (nine apo E3/7, two apo E4/7). Gene frequencies of apo E5 and E7 were 0.004 and 0.009, respectively. Significant differences in cholesterol levels were not observed among apo E3/5, E3/7 and E3/3.

A 11

PATERNITY EXCLUSION CASES BASED ON BLOOD-GROUP SYSTEMS IN OUR LABORATORY.

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Nineteen of ninety-eight cases were denied their paternity by blood group systems in our laboratory. Relation between a blood group system and number of paternity exclusion cases based on the system was as follows: ABO, four cases; MN, nine cases; P, two cases; Lewis, three cases; Rh-Hr, five cases; Duffy, two cases; Kidd, seven cases; Diego, one case. No case was denied its paternity by Kell, Lutheran or Xg blood group system. A combination of blood group systems on each paternity excluded case was as follows: four blood group systems in one case (ABO, MN, Rh-Hr & Duffy), three blood group systems in two cases (ABO, MN & P; ABO, MN & Kidd), two blood group systems in seven cases (ABO & MN, one case; MN & Lewis, one case; MN & Lewis, two cases; Lewis & Kidd, two cases; Kidd & Diego, one case), and one blood group system in nine cases (MN, three cases; Rh-Hr, two cases; Duffy, one case; Kidd, three cases).

A 12

HETEROGENEOUS EXPRESSION OF BLOOD GROUP A-DETERMINANT IN A HUMAN GASTRIC CANCER CELL LINE DERIVED FROM BLOOD GROUP A INDIVIDUAL, Hisao TAKIZAWA¹, Yoshihiko KOMINATO¹, Takashi FUJIKURA¹, Kouichiro KISHI², ¹Department of Legal Medicine, Toyama Medical and Pharmaceutical University, Toyama city, 930-01 Japan, and ²Department of Legal Medicine, Fukui Medical School, Matsuoka, 910-11 Japan

Human gastric cancer cell line MKN 45 was derived from a tumor of blood group A individual and known to express large quantities of blood group A-antigen. We found that MKN 45 cells donated from Japanese Cancer Research Resources Bank consisted of A-antigen positive cells (20%) and A-antigen negative cells (80%) using immunofluorescence. After limiting dilution, wild type and mutant cells were cloned in regard to the expression of cell surface A-antigen. By ELISA, A-antigen was shown in the cell extracts of the wild type cells, but not in those of the mutant cells. However, the blood group A-gene-specified transferase activities of the mutant cells were almost same as those of the wild type cells.

A 13

MONOCLONAL ANTIBODIES AGAINST NUCLEAR PROTEINS ASSOCIATED WITH HUMAN CHROMOSOME 21. (Ryoji YAO and Kazukiyo ONODERA Dept. Agricul.Chem., Univ.Tokyo, Tokyo)

Two monoclonal antibodies(Mabs), YO-1 and YO-3, against proteins affected by human chromosome 21 were obtained using hamster/human hybrid cells, containing human chromosome 21 as its only human chromosome, as an antigen. It was demonstrated that both YO-1 and YO-3 recognized the same protein with 32KDa in molecular weight. The 32KDa protein was specific to the hamster/human hybrid cells and did not exist in the parent hamster cells. It was also demonstrated that the 32KDa protein was localized in nucleus. Since YO-3 cross reacted with the protein with 23KDa in molecular weight that existed in both the hamster/human hybrid cells and the parent hamster cells, the epitope recognized by these two Mabs was different. Because these two Mabs recognize nuclear proteins associated with human chromosome 21, these Mabs will be useful tools to investigate not only the function of human chromosome 21 but also the function of a nuclear protein in terms of the pathogenesis of Down syndrome.

A 14**A cDNA CLONE SPECIFICALLY EXPRESSED IN 2 PATIENTS WITH TUBEROUS SCLEROSIS.** Tetsuo TAKAHASHI[#], Yasumasa ISHIBASHI^{*}, Kazukiyo ONODERA[#].

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Tuberous sclerosis (TS) is one of human dominantly inherited diseases. A cell line was established from angiofibroma of a patient with TS (22 years old, female) and it was designated TS cells. Biochemical study of TS cells revealed that they retained the properties of fibroblasts but expressed glial fibrillary acidic protein and 55kd unknown phosphoprotein. These results indicate that the gene expression of TS cells is abnormal. Therefore we constructed cDNA library from TS cells and isolated 5 cDNA clones which were specifically expressed in TS cells. Among them, pLMM1 was the most interesting clone. We also constructed cDNA library from brain tumor of a patient with TS (4 years old, male) and detected a cDNA clone hybridized with pLMM1 in this library. DNA sequence study of pLMM1 and this clone will contribute to understanding about TS.

A 15

Chromosome assignment of cDNA on chromosome 21(21q21).
Mitsuru EBIHARA, ^{*}Hisashi HAGIWARA, ^{*}Hiroyuki IKEDA, ^{*}Hiroshi NAKAI and Kazukiyo ONODARA (Dept. Agricul. Chem., Univ. Tokyo.,
^{*}Dept. Pediatr., Tohoku Univ.)

We have developed the method for effective screening of human chromosome 21 specific cDNA clones constructed from of human-hamster hybrid cells. 48 clones were successfully obtained. The efficiency of this screening was 100 times higher as compared to the former screening method such as plaque hybridization. Our method enabled us to screen for whole cDNA clones encoded by human chromosome 21, if independent clones of approximately 10⁶ were screened. However, it doesn't seem that 2Fu⁺ cells express the whole genes containing that of human chromosome 21. It will be necessary that several kind of cDNA libraries, for example human X mouse, were screened by using many kind of DNA fragments as probes. We could obtain C19 clone and many cDNA clones which were hybridized with human chromosome 21. We could not find any homologous sequence to C19. This implied that C19 encodes a new gene that had not been reported and the function of this gene is not known. C19 was expressed in a few tissue, and strongly expressed in brain the patient with Down syndrome showed the mental retardation, the fact that the region of C19 was mapped to Familial Alzheimer's disease region (21q21) has attracted our attention. It is now possible to deduce the sequence of peptide from DNA sequence of C19 and by making an antibody against synthetic peptide, it is also possible to identify the gene product of C19. Experiment along this line will contribute to understanding Alzheimer's Disease.

A 16

ISOLATION OF CHROMOSOME 21-SPECIFIC cDNA CLONES EXPRESSED IN THE BRAIN OF ALZHEIMER'S DISEASE. Yagushi KAJII¹, Hisashi HAGIWARA², Hiroiyuki IKEDA², Hiroshi NAKAI² and Kazukiyo ONODERA¹.
¹Dept. Agricul. Chem., Univ. Tokyo and ²Dept. Pediatr., Univ. Tohoku.

Antisera against 2Fu^r, a human/rodent somatic cell hybrid containing only 21q as human chromosome, were obtained. A human brain cDNA library from a patient of Alzheimer's disease was screened using one of the antisera and 25 clones in the 3×10⁵ clones were isolated. Each cDNA insert was amplified by PCR and dot-blotted on nylon membrane. The membrane was hybridized with ³²P-labelled genomic DNA from 2Fu^r or CHO (the background of 2Fu^r) and 4 of 25 clones were assumed to be on the chromosome 21. The 4 clones were further analyzed by Southern hybridization and 3 of 4 clones were shown to be single copy sequences on 21q. The efficiency of screening was 10⁻⁵. Chromosomal assignment of each cDNA insert is now under investigation.

A 17

EXPRESSION OF THE CLASS II I-A^k PREVENTS THE DEVELOPMENT OF INSULITIS IN NOD TRANSGENIC MICE.

Jun-ichi MIYAZAKI, Toru MIYAZAKI, Masashi UNO, Masahiro UEHIRA^{*}, Hirofumi NISHIMOTO^{*} and Ken-ichi YAMAMURA (Inst. for Med. Genet., Kumamoto Univ. Med. School; ^{*}Shionogi Research lab.)

The non-obese diabetic (NOD) mouse is known as an excellent model for human insulin dependent diabetes mellitus (IDDM), which is characterized by autoimmune insulinitis. Genetic studies have shown that two genetic loci, one linked to MHC and the other to the Thy-1 locus, are involved in the development of insulinitis. The NOD mouse has two unique features in the MHC class II, that is, the absence of I-E and the presence of the unique I-A^{NOD}, in which Asp at position 57 of the beta chain is replaced by Ser. This feature is also found in the HLA-DQ chain of many Caucasians with IDDM. To examine the role of the unique I-A^{NOD}, we produced transgenic NOD mice expressing I-A^k or I-A^k:57 Ser in which Asp at position 57 of the beta chain is replaced by Ser. The analysis of these mice show that expression of I-A^k prevents the development of insulinitis, indicating that not only the absence of I-E but also the presence of the unique I-A is indispensable for the development of insulinitis. In addition, the results show that the single amino acid substitution of Asp with Ser at position 57 of the beta chain is not sufficient for the susceptibility to insulinitis.

A 18

ANALYSES OF FACTORS THAT AFFECT AMYLOID DEPOSITION IN FAP TRANSGENIC MICE. Fumi TASHIRO¹, Shoji WAKASUGI¹, Shigehiro YI², Shuichiro MAEDA³, Kazunori SHIMADA³ and Ken-ichi YAMAMURA¹(¹Inst. Med. Genet., ²Dept. Pathol., ³Dept. Biochem., Kumamoto Univ. Med. School, Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder characterized by extracellular deposition of amyloid fibrils and by peripheral and autonomic nerve involvement. We have produced transgenic mice by introducing the human mutant transthyretin (TTR) gene which is considered the main cause of this disease, and have shown amyloid deposition in these mice. To further analyze the mechanisms of FAP, we investigated other factors of amyloid deposition. First, the transgenic mouse carrying the gene of human serum amyloid P component (SAP), which is involved in amyloid of FAP, was mated with the TTR transgenic (MT-hTTR) mice to make the mouse carrying both the human TTR and SAP genes. Analyses of amyloid deposition showed that the human SAP didn't stimulate the deposition of amyloid. Secondly, to examine the environmental factor, the amyloid deposition under SPF was compared with that under non-SPF. Until 18 months of age, no amyloid deposition was observed under SPF, suggesting the importance of factor(s) besides the mutant gene.

A 19

TRANSCRIPTIONAL REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR GENE IN HUMAN TUMOR CELL LINES. Shinobu GAMOU¹, Yukio SHIMOSATO², Nobuyoshi SHIMIZU¹ (¹ Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo. ² Pathol. Div., Natl. Cancer Ctr. Res. Inst., Tokyo)

Most cell lines derived from small cell lung carcinoma (SCLC) do not express epidermal growth factor receptor (EGFR) mRNA. A variant with altered morphology, AD320, was isolated from the SCLC cell line Lu134 by treatment with 5-azacytidine. EGFR mRNA and EGF binding activity could be detected in the variant. Using this variant, we investigated the regulatory mechanism of EGFR gene expression and the involvement of protein phosphorylation. Several cytosolic proteins were phosphorylated and EGFR mRNA accumulated after EGF treatment in AD320 cells. A potent tumor promoter, 12-O-tetradecanoylphorbol-13-acetate, also induced the phosphorylation of a dozen cytosolic proteins as well as the accumulation of EGFR mRNA in AD320 cells. Although Lu134 cells possessed a similar level of protein kinase C as AD320 cells, the phosphorylation of an acidic 90 kDa protein was solely induced in Lu134 cells' cytosol. Thus, the EGF receptor in the variant appeared to be an active part of the trans-membrane signaling machinery, and a phosphorylation mediated-pathway, which may be involved in the regulatory mechanism of EGFR gene expression, was more active in the variant.

A 20

DEVELOPMENT OF THE HIGH-LEVEL EXPRESSION SYSTEM OF HUMAN β -GALACTOSIDASE GENE USING A RECOMBINANT BACULOVIRUS VECTOR.

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A full-length cDNA encoding human acid β -galactosidase was inserted into the baculovirus genome under transcriptional control of the viral polyhedrin gene promoter. The *Spodoptera frugiperda* cells infected with the recombinant virus expressed the β -galactosidase activity 300-fold higher than human fibroblasts. Immunoblot analysis revealed an 82-kDa protein band, which was modified in molecular size by deglycosylating enzymes; N-glycanase digest (an 80-kDa protein) and endoglycosidase H digest (80-kDa and 81-kDa), indicating that the enzyme molecule was glycosylated, partly with high-mannose type oligosaccharides. The intracellular distribution of the enzyme observed by indirect immunofluorescence staining was perinuclear or diffusely cytoplasmic, and not characteristic of lysosomes; the enzyme was secreted to the culture medium in large quantities, and not translocated to lysosomes. This expression system is applicable to study the structure and function of normal and mutant human β -galactosidases.

A 21

ミトコンドリアDNA欠失の発生機序についての考察、後藤雄一^{1,2}、宝来 聡³、埜中 征哉¹ (¹国立精神・神経センター神経研究所、²北大医学部小児科、³国立遺伝学研究所)
A STUDY FOR THE MECHANISM OF DELETIONS IN HUMAN MITOCHONDRIAL DNA, yu-ichi GOTO^{1,2}, satoshi HORAI³, ikuya NONAKA¹ (¹Natl. Inst. Neuroscience, N.C.N.P., Tokyo, ²Dep. Ped., Univ. Hokkaido, Sapporo, ³Natl. Inst. Genet., Mishima)

ミトコンドリアミオパチーの中で、外眼筋麻痺を主症状とする慢性進行性外眼筋麻痺症候群(CPEO)では、その半数以上の症例でミトコンドリアDNA(mtDNA)の欠失が認められる。演者らは、すでに30例で欠失mtDNAを確認しており、それらの内17例について欠失断端の正確な位置をpolymerase chain reactionとサンガー法を用いて決定した。17例の内16例で、断点周辺にdirect repeatsか、類似性の高い塩基配列が存在した。この結果から、“slipped mispairingを介するrecombination”が、その発生機序として最も妥当と考えられた。また演者らは、同一部位のrepeatsが関わっている症例で、二種類の欠失が生じることを見いだした。これは、repeatや類似性の高い配列の“中”で交叉が起こることを示すものと考えられた。一方、演者らは断点周辺に何ら類似性のない一症例を経験し、“slipped mispairingを介するrecombination”によらない欠失のあることを見いだした。以上のように、大部分が“slipped mispairingを介するrecombination”にてmtDNAの欠失が生じるが、実際の交叉が起こる位置はrepeatの中にあること、また、他の機序でも欠失が起こりうることを示した。

A 22

MOLECULAR BASIS OF HUMAN N-ACETYLATION POLYMORPHISM AND ITS CLINICAL APPLICATION. Masami MASHIMO, Tomokazu SUZUKI (Dept. Clin. Genet., Med. Inst. Bioregulation, Kyushu Univ., Beppu) and Takeo DEGUCHI (Dept. Molecular Neurobiol., Tokyo Met. Inst. Neurosciences, Fuchu)

We reported a simple, more accurate method by DNA analysis for the diagnosis of human N-acetylation polymorphism. Firstly, acetylator phenotypes were determined on 59 healthy volunteers by oral isoniazid loading test, and they were classified as rapid (n=26), intermediate (n=29) or slow (n=4) acetylators. Then Southern blot analysis was performed with genomic DNAs from 29 of 59 volunteers using the 32P-labelled cDNA for polymorphic arylamine N-acetyltransferase in human liver. Three types of N-acetyltransferase gene were identified, and the combination of these three genes generated five genotypes. The correlation between phenotypes and genotypes was as follows; rapid acetylators belonged to genotype I (n=10, homozygote of gene 1). Intermediate acetylators belonged to genotype I (n=2), II (n=2, heterozygote of gene 1 and 2) or III (n=12, heterozygote of gene 2 and 3). Slow acetylators belonged to either genotype IV (n=1, heterozygote of gene 2 and 3) or V (n=2, homozygote of gene 3). Consequently the genotyping method reported here is applicable to the diagnosis of N-acetylation polymorphism, particularly to the identification of slow acetylator which is extremely important to clinical medicine.

A 23

MOLECULAR DELETION IN THE INSULIN RECEPTOR ALPHA SUBUNIT GENE IN A PATIENT WITH LEPRECHAUNISM. Hidefumi TONOKI, Nobuo MATSUURA, Masanori NAKANISHI, Susumu IIZUKA, and Kenji FUJIEDA. Dept. of Pediatrics, Hokkaido University School of Medicine, Sapporo and Kuchian Kosei Hospital, Kuchian.

Molecular genetics and endocrinological studies of a patient with severe insulin resistance with a syndrome of Leprechaunism are reported. Southern blot studies of genomic DNA from the patient revealed an 1.3 kb genomic deletion in alpha subunit gene of the human insulin receptor gene in the maternal allele. As little is known at genomic structure of this region, restriction map was made with several endonucleases encompassing from exon 4 to exon 7. Southern blot hybridization study with exon specific probes made by PCR method revealed that the exon 5 is deleted in the maternal allele. Binding assay of insulin to Epstein Bar virus transformed lymphoblasts of the patient demonstrated an extreme reduction of binding activity. Although the mutation in the paternal allele is not identified, the deletion of exon 5 in the alpha subunit is considered to contribute to decrease in number of insulin receptor at cell surface or reduction of insulin binding.

A 24

THE GENETIC ANALYSIS OF WERNER'S SYNDROME. Jun NAKURA¹ Tetsuro MIKI¹ Tomohiro KATSUYA¹ Yuji TAKEMOTO¹ Kenichiro FUKUCHI¹ Hidetsugu KUZUYA² Yoshisada FUJIWARA³ and Toshio OGIHARA¹ (1Dept. Geriatric Med., Osaka Univ. Med. Sch., Osaka, 2Dept. Internal Med. II, Kyoto Univ. Med. Sch., Kyoto, and 3Dept. Radiation Biophysics, Kobe Univ. Med. Sch., Kobe, Japan)

Werner's Syndrome (WS) is a rare autosomal recessive disorder characterized by the appearance of features of premature aging in a young adult. Skin fibroblasts from WS patients demonstrated slow growth, reduced life span in vitro and mutator phenotype. The genetic defect in WS is unknown. The incidence of WS progeny of a first-cousin marriages in Japan is higher than that in Caucasian countries, because consanguineous marriage is not rare in Japan. We have studied nine WS patients from first-cousin matings derived from seven different families and examined the status of genetic heterogeneity among WS patients. All of them satisfied the diagnostic criteria made by the research group in Japan. We excluded the phenotypic heterogeneity among these unrelated persons. The principal strategy for mapping the WS gene is based on the homozygosity mapping method. We are employing VNTR DNA markers as probes for these studies. Seven of nine patients demonstrated heterozygosity for D2S44(pYNH24). Eight of nine patients demonstrated heterozygosity for D10S26(pEFD 70.2). Therefore the regions nearest to D2S44 and D10S26 were excluded.

A 25

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) OF PCR-AMPLIFIED DNA FRAGMENTS (PCR-DNA) FOR DETECTION OF VARIATIONS IN DNA.

Norio TAKAHASHI, Chiyoko SATOH, Hideo OMINE, Akiko MIURA, Jun-ichi ASAKAWA. (Dept. Genet., RERF, Hiroshima)

The search for variations in DNA to evaluate the genetic effects of atomic bomb radiation requires a technique which can examine as many base pairs (bp) as possible at one time. DGGE of PCR-DNA appears to be effective for this purpose. Now we are accumulating data from DGGE of PCR-DNA using the human factor IX gene (FIX). Variations observed in FIX of 124 Japanese were presented. In the first experiment, a region between positions -1218 and -236 from the proposed initiation transcription site of FIX was amplified. The DNA was cleaved into two segments by *TaqI*, and examined. Two polymorphic C to T substitutions were observed (one occurred at -1186 and the other at -698). In the second experiment, a 1618 bp fragment, obtained by amplification of the region between -115 and 1503, was cleaved by *EcoT22I* and *XbaI* into three segments and analyzed. A polymorphic A to G substitution at 192 was observed, and a C to A substitution at 181 was detected in one individual. The 1618 bp fragment also was analyzed following *HaeIII* digestion. An A to G substitution was found at 709. Because of its high detection rate of variations, we believe DGGE of PCR-DNA is the most effective method for population studies.

A 26

DETECTION OF VARIATIONS IN mRNA EMPLOYING DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) COMBINED WITH POLYMERASE CHAIN REACTION (PCR). Chiyoko SATOH, Norio TAKAHASHI, Jun-ichi ASAKAWA, Keiko HIYAMA, Hideo OMINE, Akiko MIURA. (Dept. of Genet., RERF, Hiroshima)

DGGE is a very efficient technique for screening for variations in genomic DNA when it is combined with PCR. In order to apply the PCR-DGGE in the detection of variations in mRNA, a model experiment was carried out using a hereditary phosphoglycerate kinase 1 (PGK1) variant with the activity less than 1/2 of the normal value which was detected in our previous screening for mutations in children of atomic bomb survivors. RNA was extracted from cell lines established from B-cells of the propositus and cDNA was synthesized. A target sequence of 1668 bp between positions -57 and 1611 (the first nucleotide in the initiation codon is 1) was amplified and digested with *StuI* and *SspI*. Resulting 3 fragments of 655, 650 and 363 bp were examined with DGGE. An abnormal mobility was observed in the 650 bp-fragment. Sequence analysis revealed a G to T substitution at the 8th position of the fragment corresponding to the third nucleotide of the codon 201 that produces a Glu to Asp substitution. Studies of 2 other variants with activities two times higher than the normal value are under study. The DGGE-PCR seems to be practical to detect variations in mRNA in the population studies.

A 27

SEX IDENTIFICATION OF FORENSIC SAMPLES BY POLYMERASE CHAIN REACTION. Atsushi AKANE, Kazuo MATSUBARA, Setsunori TAKAHASHI, Hiroshi SHIONO (Dept. Legal Med., Shimane Medical Univ., Izumo), Yutaka NAKAHORI, Satoko SEKI, Shigeo NAGAFUCHI, Masao YAMADA and Yasuo NAKAGOME (Dept. Congen. Abnormal. Res., Natl. Child. Med. Res. Cent., Tokyo).

Sex identification of forensic samples (bloodstains and putrefied body) was performed by amplifying X-Y homologous region or Y-specific repeating units by Polymerase Chain Reaction (PCR) technique. Analysis by PCR on X-Y homologous region encoding "amelogenin" gene revealed both Y- and X-specific bands, which were amplified simultaneously from the common primers, resulting in fragments of different length. Since the detection of X-specific band indicated the success of the procedures, analysis of this locus was one of the most reliable methods for sex identification. However, more than 250 ng/tube of DNA sample was required for the analysis, since the locus only includes the single copy gene. On the other hand, DYZ1 locus which is constructed by thousands of copies of repeating units pHY10 could be amplified from less than 0.25 ng/tube of DNA sample. Even when DNA from a putrefied tissue was analyzed, DYZ1 locus could be detected by PCR.

A 28

MICROCLONING OF THE DM region (19q13.2) EXCISED FROM A SINGLE CHROMOSOME BY LASER LASER MICROBEAM AND PCR.

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Direct cloning of the target region on a chromosome makes a rapid analysis about a responsible gene for a disease without time consuming. To clone the gene of myotonic dystrophy (DM) located on chromosome 19q13.2, we developed a new method. Exception of range from 50 to 80% distal of 19q, all chromosome as well as nuclei in a metaphase preparation were burned down by argon ion laser. One chromosome fragment prepared as above was recovered from a slide glass in 2µl of extraction buffer. After proteinase K and PMSF treatment, genomic DNA recovered was amplified by PCR method using Alu core sequence (TC65) as a primer. PCR products were ligated to SmaI site of pUC118 and transformants and average size of inserts is 200bp-300bp. PCR products derived from microdissection region of chromosome 19 was ascertained by chromosome in situ hybridization (CISH). The cDNA OF ERCC1 hybridized PCR products. This suggests that DNA library generated with PCR products might contain fragment of candidate gene of DM.

A 29

DNA ANALYSIS FOR *APRT* * *J* DETECTING A SIGNAL OF CHEMILUMINESCENCE

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Adenine phosphoribosyl transferase (APRT) deficiency is a common genetic disorder among Japanese. Most of patients are homozygote having a mutant APRT gene (*APRT* * *J*). *APRT* * *J* gene has a substitution of T to C at position 2069 in exon 5, and results the low affinity enzyme for phosphoribosylpyrophosphate.

We reported a hypersensitive dot blot assay for *APRT* * *J* mutation using a competitive hybridization of allele specific oligonucleotide and a direct chemiluminescent substrate for alkaline phosphatase, AMPPD(1,2-dioxetane).

In this method, we successfully amplified the target DNA without extracting, and detected that mutation as sensitive as ³²P system. We confirmed this method was applicable assay system for detection of other point mutation.

A 30

Isolation of transcribed sequences from human Xq24-qter. O. Onodera, H. Kobayashi, T. Miyatake and S. Tsuji. (Department of Neurology, Brain Research Institute, University of Niigata. 1 Asahimachi Niigata city, 951 Japan)

The loci of many diseases have been assigned to the region of Xq24-qter. Isolation of expressed genes on Xq24-qter will be useful for identifying the disease genes. Recently P. Liu *et al.* developed a new strategy using heterogenous nuclear RNA as templates for cDNA synthesis (Science 246:813-815, 1989). We took the strategy for isolating transcribed sequences from Xq24-qter.

Poly(A)⁺ RNA was isolated from a human-chinese hamster ovary cell hybrid, X3000-11.1 (kindly provided by Dr. Nussbaum) containing the region of human Xq24-qter. Four different hexamers complementary to the 5' splice sites of introns were used as primers for cDNA synthesis and we constructed a lambda gt11 cDNA library. The total number of independent cDNA clones was 6×10^6 , of which approximately 1100 clones (0.02%) hybridized to the total human genomic DNA probe. This strategy should give us many candidate genes for diseases mapped to the region.

A 31

CHROMOSOME MAPPING OF HUMAN GENES BY NONISOTOPIC *IN SITU* HYBRIDIZATION: TECHNICAL PROBLEMS AND THEIR SOLUTIONS. Ei-ichi TAKAHASHI, Tada-aki HORI (Natl. Inst. Radiol. Sci., Chiba), Yusuke NAKAMURA (Cancer Inst., Tokyo) and Takeshi SENO (Natl. Inst. Genet., Mishima)

Recently developed nonisotopic *in situ* hybridization techniques have overcome some of the problems associated with the use of isotopes; long exposure times, uncertain localization of signals due to grain scatter and high background "noise". The current nonisotopic techniques with biotin-avidin system also have some problems to be solved; 1) direct mapping on banded chromosomes, 2) detection of small single copy genes (<5kb) and 3) elimination of "Alu-noise" on chromosomes. The systems we have developed and modified are as follows; 1) direct mapping on replicated prometaphase R-bands, 2) amplification procedure of signals and 3) preincubation with total human DNA. By this R-banding technique, one can analyze the molecular structures of chromosomal bands: R-positive bands; early-replicating, GC-rich sequences, housekeeping genes, R-negative bands; late-replicating, AT-rich sequences, tissue specific genes. These three systems can be applied for the clones containing more than 1.1kb.

A 32

The human thymidylate synthase gene is located on the telomeric R-band of the short arm of chromosome 18, 18p11.32

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(¹Div.Genet., Natl.Inst.Radiol.Sci. ²Lab.Mutagenesis, Natl.Inst.Genet.)

Thymidylate synthase (TS) gene is a key enzyme in DNA replication. In thymidylate stress conditions, various chromosome instabilities including fragile site expression are shown to be induced. There have been two contradicting reports on the location of the TS gene; 18pter-18q12(Hori et al.,1985) and 18q21-qter(Nussbaum et al.,1985). By applying nonisotopic in situ hybridization method(Takahashi et al.,1990) with biotinylated cDNA and genomic probes of human TS gene, we have confirmed our previous mapping results on somatic cell hybrids between thymidine-auxotrophic mouse mutant cells and fragile X human cells and further localized the TS gene to the telomeric R-band of 18p11.32. The TS gene is a typical housekeeping gene. The most of housekeeping gene are shown to be GC-rich in their DNA sequences, located on the R-band and replicate in early half of the S phase. The localization of the TS gene to the R-band support this notion. The TS gene provides a useful telomeric anchor point for linkage mapping on the short arm of human chromosome 18.

A 33

GENE MAPPING WITH SORTED CHROMOSOMES SEPARATED BY CELL SORTER. Hisashi HAGIWARA, Kaoru TAKEDA, Hiroyuki IKEDA, Hiroshi NAKAI and Keiya TADA (Dept.of Pediatrics, Tohoku University School of Medicine, Sendai)

We have assigned the gene locus of human liver bilirubin UDP-glucuronosyl-transferase(UDPGT) to chromosome 1 or 2 by a spot-blot hybridization to flow-sorted chromosome. Sorted chromosomes were prepared from metaphase of human lymphoblast line GM00130B(46,XY) or GM00131(46,XX) by the polyamine/digitonin method, stained by Hoechst 33258, and sorted directly into spot on filter discs. In spot-blot hybridization we used a ³²P-labeled cDNA(HLUG25) probe purified by Brian BURCHELL. This was consistent with our result of the mapping by in situ hybridization.

A 34

MAPPING FIFTEEN SITE SPECIFIC GENES OF HUMAN CHROMOSOME 21. Hiroshi NAKAI, Kaoru TAKEDA, Hisashi HAGIWARA, Hiroyuki IKEDA and Keiya TADA (Dept. of Pediatr., Tohoku Univ. School of Medicine, Sendai)

Eleven site specific DNA fragments, cloned by cosmid vector(Watkins), three cDNA and one Alu-PCR gene product were assigned by *in situ* hybridization using tritium labeling. As control samples, SOD-1 cDNA(Groner) and human ribosomal DNA(Muramatsu) were used and also non-radioactive *in situ* hybridization was applied in success as well as autoradiography.

SOD-1 was localized to 21q22.1. R-DNA was on short arms p12 on acrocentric chromosomes. Fluorescent method also worked very well as for the repeating r-DNA. One chromosome 21 specific anonymous DNA fragment was assigned near centromeric region of the chromosome. Two DNAs at 21q11, three were assigned to 21q11-q21, q21, q21-q22.1 respectively. and five DNA fragments were to 21q22.3. As for three cDNAs, each were localized on 21q11.2-q21, q21 and q22.3 respectively. One Alu-PCR product gene of a large DNA was assigned to 21q21-q22.1, that was cloned by yeast artificial chromosome.

A 35

ESTABLISHMENT OF LYMPHOBLASTOID CELL LINES WITH STRUCTURAL CHROMOSOME ABNORMALITIES, AND THEIR USEFULNESS FOR REGIONAL GENE MAPPING.

Tatsuro IKEUCHI¹, Kohtaro YAMAMOTO², Fumiko SAITO¹, Eiko ARAI¹, and Akira TONOMURA¹ (¹Dept. Genet., ²Dept. Virol. Immunol., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo 113)

EB virus-transformed lymphoblastoid cell lines (LCLs), grown in suspension with high cell density, are of highly practical value in experimental human genetics. In particular, LCLs with chromosome rearrangements are the valuable sources for physical gene mapping studies. In the past few years, we have established a total of 60 LCLs derived from individuals with structural chromosome abnormalities. They include 26 deleted (interstitially and terminally) and 9 duplicated regions, and 40 translocation breakpoints. All the chromosomes, except Nos.8 and 16, were involved in the above rearrangements. Some of the LCLs were actually applicable to the following refined regional gene mapping: PGM1 (1p22.1), D13S21 and D1322 (13q14.1-q14.2), HMC syndrome (1q31.2 or 7p15.1-p15.3), anhydrotic ectodermal dysplasia (Xq21-q13), neurofibromatosis-2 (22q11.23-q12.2), etc. We emphasize the special importance to establish in Japan also the storage system for the chromosome mutations which are in a sense the valuable genetic resources.

A 36

CHROMOSOME ASSIGNMENT OF HUMAN GENES BY USING PCR. Hisao UHEYAMA¹, Kiyoshi KUROKAWA¹, Michihiro YOSHIDA², Mitsuo OSHIMURA³, and Naotoshi KANDA⁴ (¹Dept. Med. Biochem., Shiga Univ. Medi. Sci., Otsu, ²Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., ³Dept. Cell Technol., Fac. Med., Tottori Univ., Yonago, ⁴2nd Dept. Anat., Tokyo Women's Med. College)

Chromosome mapping using rodent-human somatic cell hybrids has been done by Southern blot with a specific probe for the gene of interest. We examined whether it can be done by PCR (polymerase chain reaction).

First we synthesized amplification primers for human LDL receptor and human δ globin genes. By the PCR method, we could assign these genes to chromosomes 19 and 11, respectively, being in good agreement with the previous data.

Next we examined three human genes the chromosome loci of which are not known yet. By the PCR method, we could determine the loci of human enteric type smooth muscle actin, human preangiotensinogen, and human glial fibrillary acidic protein genes to be on chromosomes 2, 1, and 17, respectively.

The method has several advantages over the Southern blot method. (i) It needs little amount of hybrid cell DNA. (ii) It takes only 2 days to obtain results, when the primers are ready. (iii) It does not require to have gene-specific probes, though it requires the nucleotide sequence data. (iv) It avoids the use of radioisotopes such as ³²P.

A 37

HETEROMORPHISMS OF AG-STAINED NUCLEOLAR ORGANIZER REGIONS IN THE HIROSHIMA F₁ POPULATION. Yoshiaki KODAMA, and Akio A. AWA (Dept. of Genetics RERF, Hiroshima).

In the present study, 148 children of A-bomb survivors (89 exposed and 59 controls) were studied for the variation of nucleolar organizer regions (NORs). Ten metaphases from whole blood cultures, stained in combination with silver staining and G-banding methods, were analyzed in each individual. The absolute size of the Ag-NORs was measured in the cell which showed a representative staining pattern using a NIKON modular system for semiautomatic quantitative evaluation of images. It was found that the Ag-stained chromosomes did not occur randomly, and the inter-chromosomal distribution of Ag-stained deposits showed a consistent pattern for each individual. No significant difference was observed between proximally and distally exposed groups in terms of both mean and modal numbers of Ag-NORs, as well as in the size of Ag-stained deposits. In both groups, Ag-NORs in chromosomes 13 showed strong staining intensity, while chromosomes 22 showed weak intensity. The modal number of Ag-positive chromosomes per individual varied from 5 to 10. The absolute size of Ag-NORs per Ag-positive chromosome was inversely related to the modal number of Ag-NORs; i.e., there was no inter-individual difference in the size of Ag-NORs per cell. This finding would suggest the existence of a compensatory mechanism by which the NOR-activity is regulated.

A 38

GENES AT THE BREAKPOINTS OF CHROMOSOMAL REARRANGEMENTS: DEVELOPMENTAL ARREST OF CHINESE HAMSTER EMBRYOS HOMOZYGOUS FOR REARRANGEMENTS. Shin-ichi SONTA (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai, Aichi), Tadashi IIDA (Nagoya City Univ. Med. Sch., Nagoya)

Using Chinese hamsters heterozygous and homozygous for X-ray-induced chromosomal rearrangements, we investigated the relationship between phenotypical abnormalities and the rearrangements. Forty-three Chinese hamster stocks with "balanced" autosomal rearrangements, 5 inversions and 38 reciprocal translocations, were used. Male and female heterozygotes for the rearrangements were all fertile and morphologically normal except for one stock with growth retardation. From crosses between male and female heterozygotes for the same rearrangements, homozygotes were obtained in 37 lines. In the remaining 6 lines (1 inversion and 5 reciprocal translocations), however, no homozygotes were obtained. The results of chromosome analyses revealed that homozygous embryos in the 6 lines arrested development at the two-cell stage, around the eight-cell stage or after implantation, respectively. The breakpoints of the rearrangements relating to homozygous lethality were different in each. We obtained viable homozygotes for other rearrangements having breakpoints in the more proximal region than those of the rearrangements relating to homozygous lethality. These results suggested that abnormal phenotypes including homozygous lethality might occur due to an influence of genes at the breakpoints.

A 39

SECOND MEIOTIC NONDISJUNCTION OF THE REARRANGED CHROMOSOMES IN A FAMILIAL RECIPROCAL 5/13 TRANSLOCATION. Mitsuo MASUNO¹, Yang CHOLSONG¹, Takashi KUWAHARA¹, Nobuo SHIMIZU¹, Tadao ORII¹, Ichiro KAWABATA², Hiroshige MIKAMO², Teruhiko TAMAYA² (¹Dept. Pediatr., ²Dept. Obstet. & Gynecol., Gifu Univ. School of Med., Gifu)

A 20-week-gestation male abortus with partial 5p trisomy and proximal 13q trisomy, 47,XY,t(5;13)(p15;q21), +der(13)t(5;13)(p15;q21) mat, is presented. Autopsy showed persistent truncus arteriosus (type I in the classification of Collet & Edwards), ventricular septal defect (membranous defect), agenesis of the ductus arteriosus, right aortic arch, dextrocardia, peritoneo-pericardial diaphragmatic hernia (left lobe of liver, stomach and spleen had herniated into the left thoracic cavity; pericardial defect was 1.1 cm × 0.5 cm), lobulation anomaly of lung (right, 2 lobes; left, 1 lobe), single umbilical artery, duplication of the left thumb with syndactyly, right palmar simian crease and bilateral prominent heel. This finding might be attributable to second meiotic nondisjunction of the rearranged chromosomes in a maternal balanced reciprocal translocation. To the best of our knowledge, there has been only one previous report of a similar error in the segregation of the rearranged chromosomes. This second meiotic malsegregation should be taken into account in the consideration of reproductive problems in carriers of balanced translocations.

A 40

DNA ANALYSIS OF 132 PATIENTS WITH SEXUAL ABNORMALITIES AND MAPPING HUMAN Y CHROMOSOME. Takashi TAMURA^{1,2}, Shigeo NAGAFUCHI¹, Yutaka NAKAFORI¹, Toshiyuki FURUSHO², Kenji FUJIEDA³, Shigeru MINOWADA⁴. (¹Dept.Cong.Abn.Res., Nat.Child.Med.Res.Ctr., Tokyo; ²Dept.Clin. Genet., Kyorin Univ. School Hlth.Sci., Tokyo; ³Dept.Pediat. Univ. Hokkaido, Sapporo; ⁴Dept.Urol., Univ.Tokyo)

A total of 132 patients with various sexual abnormalities were analysed using 16 DNA probes which potentially detect 22 different loci within the non-fluorescent region (NER) of the Y chromosome. A total of 43 patients had structurally abnormal Y chromosomes. Through deletion mapping, the NFR was divided into 20 intervals which represents phenomenal improvement from 15 in November 1989. Segments presumably responsible for short stature and azoospermia were tentatively identified. Two patients showed complicated structural rearrangements involving at least 3 breakages, respectively.

A 41

CYTOGENETIC FEATURES OF EBV-TRANSFORMED B-LYMPHOBLASTOID CELLS IN VITRO. Mimako NAKANO, Shozo IIDA, Masashi HIRAMOTO, Yoshiaki KODAMA, Kazuo OHTAKI, Akio A. AWA, (Dept. of Genetics, RERF, Hiroshima)

A pilot study was conducted prior to determining a dose-response relationship for EBV-transformed B lymphocytes in the A-bomb survivors.

Study subjects consist of two groups ; Group I from 4 healthy adult volunteers, and Group II comprising 4 distally exposed AHS survivors (0-dose group), and 9 F₁ children born to survivors. In Group I, lymphoblastoid cells were periodically stained by the immunoperoxidase technique. At 1-2 weeks after culture initiation, most of mitotic cells were IgM+ B cells, but CD3+ T cells were also present about 5% of the total mitoses. The proportion of CD3+ T cells decreased with increasing culture time, around 1% after 3 weeks of culture. Therefore, culture time of 3 weeks was selected for chromosome analysis in cultured B cells using G-banding technique on subjects in Group II. The frequencies of aberrant cells ranged from 1 to 11%. Chromosome aberrations observed most frequently were characterized by the terminal deletion. The frequency of cells with deletion involving chromosome 1 was 0.9% of the total. Of the 19 cultures from 13 donors, cells with del(1) were observed in 8 cultures (42%). Since the deletion of chromosome 1 seems to occur preferentially rather than randomly, this structural change of chromosome 1 may be associated with the immortalization of B cells in vitro.

A 42**WESTERN BLOTTING ANALYSIS FOR MALIGNANT LYMPHOMA ANTIGENS FROM CARCINOGEN-TRANSFORMED BLOOM SYNDROME CELLS, Yukimasa SHIRAISHI, Kochi Medical School, Kochi**

Membrane antigen (glyco-) proteins associated with malignant lymphoma (ML) were collected from a carcinogen-transformed Bloom syndrome (BS) B-lymphoblastoid cell line by means of the panning protocol, in which antigen cell clones which specifically react with antibodies in ML patient sera were separated on polystyrene dishes coated with IgG. Gel electrophoresis and Western blotting (WB) analyses demonstrated that antigens which characterize ML gave a single band (ML, 97kDa) and did not cross-react with sera of other cancer patients or with normal sera. The present WB procedure may be useful for diagnostic purposes. The amino acid sequence of the ML antigen band (97kDa) had 76.9% (in 13 sequences) homology to that of the variable region of the immunoglobulin (Ig) heavy chain (IgV_H) at the N-terminal end. Cell clones with ML-associated antigen had marker chromosomes involving t(6;?) (p25;?), t(9;?) (q34;?), del(10)(p13), t(12;14)(q24;q11).

A 43**BACTERIAL INFECTION, RESTRICTION ENDONUCLEASES, CHROMOSOME DAMAGE, FRAGILE SITES AND CANCER****Y.R.AHUJA**

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Bacteria, which are the source of restriction endonucleases, have been shown to cause chromosome damage. Restriction endonucleases have also been shown to be clastogenic in vitro in human and other mammalian cells. Furthermore, it has been demonstrated that the restriction endonucleases causes non-random chromosome damage which coincides with fragile sites. Fragile sites have been implicated as one of the steps in carcinogenic process. It is, therefore, proposed that restriction endonucleases, released in the human body through bacterial infection, may cause specific chromosome damage, which in turn may involve oncogene activation leading to cancer.

A 45

DERMATOGLYPHICS AND SECONDARY CREASES IN HYPOHYDROTIC ECTODERMAL DYSPLASIA

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X-linked recessive hypohydrotic ectodermal dysplasia is characterised by defective development of the skin and its appendages. The heterozygous carrier female often shows some signs, and Lyonisation is suggested by the patchy distribution of sweat pores. Data on dermatoglyphics in two affected dizygous twin brothers and their mother are presented, as an addition to the rather restricted existing literature, and details of secondary skin creases are presented for the first time. The two brothers show excessive secondary creases on all fingers and most of the palm, and the heterozygous mother resembles them but to a less extreme degree. Since the father's features are quite distinctive, it is suggested that such skin details may prove of use in the carrier detection of this condition.

A 46

CHORIOPLACENTAL HISTOLOGY OF 150 FIRST TRIMESTER SPONTANEOUS ABORTIONS WITH A CHROMOSOME ANOMALY

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In a series of 322 consecutive cultures of spontaneous first trimester abortions, we found 150 conceptuses with a chromosome anomaly. Definite histological anomalies were observed in 92/150 cases : 30/39 triploidies; 20/36 Turner syndrome 45,X; 35/60 autosomal trisomies and 7/15 chromosome rearrangements. Thus, in our series, 39 % of all embryos with a demonstrated chromosome anomaly showed a normal chorioplacental histology.

These data strongly suggest that, in a study of the etiology of abortion, both chromosome and histological studies are recommended.

A 48**CONTRIBUTIONS OF SOME X-LINKED AND AUTOSOMAL GENES ON FINGER DERMATOGLYPHS**

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Finger print patterns and ridge-counts are compared between individuals affected with either X-linked colour blindness or certain autosomal diseases and their same-sexed unaffected relatives. The objective is to search for association or linkage of dermatoglyphs with some major genes. Statistical comparisons of within and between genotypes reveal suggestions of possible linkage of finger dermatoglyphs both with X-linked genes and certain autosomal genes. These results are also supported by results of studies on inbreeding effects.

A 49

膠原病とくに PSS, PM/DM, PN の家族発生率, 山口雅也, 大田明英, 森戸文隆, 緋田めぐみ (佐賀医科大学内科), RATE OF FAMILIAL OCCURRENCE OF COLLAGEN DISEASES, Masaya YAMAGUCHI, Akihide OHTA, Fumitaka MORITO, Megumi HIIDA (Department of Internal Medicine, Saga Medical School, Saga City, Japan).

膠原病の代表格で全身性エリテマトーデス (SLE) や慢性関節リウマチ (RA) は, 少なくとも数パーセントの家族発生率を示し, 多数の研究結果から多因子遺伝形質と考えられている。しかし同じ膠原病である全身性強皮症 (PSS), 多発筋炎/皮膚筋炎 (PM/DM) や多発性動脈炎 (PN) では家族性は稀とされている。今回われわれは, これらの膠原病の家族発生率を正確に知る目的で, 国内の 8 専門施設で診療した患者の家族を retrospective に調査した。その結果, PSS 449 家系の第一度近親では PSS 1 例 (0.22%) SLE 2 例 (0.44%) RA 10 例 (2.23%) その他 1 例が見出され, PM/DM 226 家系の第一度近親中 PM/DM な 1 例もなく, SLE 2 例 (0.88%) RA 5 例 (2.21%), その他 2 例が見出されたにすぎない。また PN (59 家系) ではさらに少なく, 分類不明の膠原病 (UCTD) 1 例 (1.69%) のみであった。以上の 3 疾患では, SLE や RA と異なり家族発生が稀で, ポリジーンの期待値に合わない。この rarity の原因について考察を加えた。

A 50

TREND OF THE INCIDENCE OF DOWN SYNDROME IN KANAGAWA BIRTH DEFECTS MONITORING PROGRAM

Division of Medical Genetics, Kanagawa Children's Medical Center
Kenji KUROSAWA, Fumio TAKADA, Kiyoshi IMAIZUMI, and Yoshikazu KUROKI

The incidence of Down syndrome (DS) in the general population depends mainly on three factors: the maternal age distribution in the population, the maternal age-specific risks, and the prevalence of prenatal diagnosis followed by selective abortion. The Kanagawa Birth Defects Monitoring Program (KAMP) is one of the population based monitoring programs in Japan, started in 1981. We collected DS from KAMP between October 1981 and December 1987, and DS born during the same period from registry of the outpatient clinic of Kanagawa Children's Medical Center (KCMC). In the mothers of DS, the ratio of the age class over 35 years old was increased from 24.1 % in 1982 and 1983 to 36.8 % in 1986 and 1987. The incidence of DS in the same class was also increased from 21.5 to 25.1 for 10,000 births. The total incidence estimated from the DS cases registered in the outpatient clinic of KCMC was not decreased in Kanagawa Prefecture. It is suspected that the prenatal diagnosis at the present state does not affect the incidence of DS in Japan.

A 51

CYTOGENETIC STUDIES IN 2 CASES OF NEUROFIBROMATOSIS TYPE 2. Eiko ARAI¹, Tatsuro IKEUCHI¹, Kohtaro YAMAMOTO², Shinji KARASAWA³, Akira TAMURA³, Mitsushiro KIDA⁴, Akira TONOMURA¹. (¹Dept. Genet., ²Dept. Virol. Immunol., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo, ³Dept. Neurosurg., ⁴Dept. Pediatr., Teikyo Univ., Tokyo)

Neurofibromatosis has two clinically distinct forms: NF-1 (von Recklinghausen disease) characterized by the tumor development in peripheral nervous system, and NF-2 (bilateral acoustic neurofibromatosis) associated with the central nervous system tumors. Both are known as autosomal dominant disorders. The NF-1 gene has been located to chromosome 17q11.2 and the NF-2 gene to chromosome 22q11-22q13.1 regions. We performed chromosome analyses on both peripheral blood and primary tumor samples obtained from two NF-2 patients. Case 1, 24-year-old female, showed a constitutional reciprocal translocation of 46,XX,t(4;22)(q11;q11.2). The tumor chromosomes were examined in short term cultures and the same translocation was observed, but no other abnormality was found. Although her father was also a carrier of the same translocation, clinically no NF-2 has been developed. Case 2, 53-year-old female, had a normal karyotype in both peripheral lymphocytes and cultured tumor cells. In order to attempt molecular analyses of the breakpoints, lymphoblastoid cell lines were established by EB virus-mediated transformation from the peripheral blood of the two translocation carriers.

A 52

LOW MOLECULAR WEIGHT COMPOUNDS RESPONSIBLE FOR HYPERSENSITIVITY TO BLEOMYCIN IN AT B-LYMPHOBLASTOID CELLS, Ming-jie LI and Yukimasa SHIRAISHI, Kochi Medical School, Kochi

Co-cultivations were applied to clarify the low molecular weight compounds through gap-junctions on chromosome abnormalities. In separate culture condition the ataxia telangiectasia (AT) cells and Bloom syndrome (BS) cells were sensitive to neocarzinostatin (NCS) or bleomycin and mitomycin C (MC) respectively, though the normal cells were not sensitive to NCS and MC. When AT cells were co-cultured with BS or normal cells and treated with NCS, the chromosome breaks per cell in BS and normal cells increased from 0.39 to 1.01 and from 0.29 to 1.11 respectively, whereas the chromosome breaks per cell in AT cells decreased from 1.58 to 1.19. The elevated sensitivity to NCS in BS and normal cells after co-culture with AT cells suggests that there are some low molecular weight compounds (responsible for the hypersensitivity to NCS) which are transferred from AT cells to BS or normal cells. The sensitivity to MC in BS cells is also discussed in relation to the transfer of thymidine (dT) pool from mutant cells with high dT pool.

A 53

REPLICATION ASYNCHRONY BETWEEN HOMOLOGS 15q11.2: CYTOGENETIC EVIDENCE FOR GENOMIC IMPRINTING.

Yoshinori IZUMIKAWA, Kenji NARITOMI and Kiyotake HIRAYAMA (Dept. of Pediatr., Univ. of the Ryukyus, Okinawa)

Replication kinetics of Prader-Willi syndrome critical region (15q11.2) was investigated in seven normal healthy adult females using RBG replication bands. Replication asynchrony between homologs 15q11.2 was identified constantly in about 40% of cells in all individuals. It was limited in the stages which Xp22, Xp11, Xq13 and Xq24/26 were visible in late-replicating X. This asynchrony suggested presence of overlapping of replication timing between 15q11.2 and early replicating R-bands of late X in some proportion of cells, as well as difference of replication timing between homologs probably related to genomic imprinting, which has been suggested as a pathogenetic basis of Prader-Willi syndrome. As a result of an analysis of asynchronous and synchronous cell proportions in each replication stage, two types of cells deduced when 1:1 methylation mosaicism of genomic imprinting was assumed and applied. First type was the cells with normal replication in one homolog and delayed replication in the other. Second type was the cells with normal replication in both homologs. Our result was considered as a cytogenetic evidence of methylation mosaicism for mammalian genomic imprinting.

A 54**CHROMOSOMAL ASSIGNMENTS OF THE HUMAN ENDOTHELIN FAMILY GENES; ENDOTHELIN-1 (EDN1) TO 6p23-p24, ENDOTHELIN-2 (EDN2) TO 1p34, AND ENDOTHELIN-3 (EDN3) TO 20q13.2-q13.3.**

Tadao ARINAMI, Mikiyo ISHIKAWA, Akihiro INOUE, Masashi YANAGISAWA, Tomoh MASAKI, Hideo HAMAGUCHI (Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba), Michihiro C. YOSHIDA (Chromosome Research Unit, Hokkaido University, Sapporo)

Endothelin is a peptide consisting of 21 amino acids with various biological functions including potent, long-acting vasoconstriction. There is an endothelin gene family comprising 3 types of endothelin, i.e. endothelin-1, -2, and -3 (ET-1, -2, and -3). Chromosomal localizations of the ET-1 (EDN1) and ET-3 genes (EDN3) in human were reported by Bloch et al. (1989), who assigned EDN1 to chromosome 6 and EDN3 to chromosome 20 by analysis of genomic DNA from human-mouse somatic cell hybrids. We reported here assignments of the human endothelin family genes by in situ hybridization of a tritium-labeled human preproendothelin cDNA of each gene and, in ET-2, Southern blot analysis of DNAs from human-mouse hybrid panels using the same probe labeled with phosphorus32-labeled-dCTP. EDN1, EDN2, and EDN3 were assigned to 6p23-p24, 1p34, and 20q13.2-q13.3, respectively.

B 1**MOLECULAR ANALYSIS OF XERODERMA PIGMENTOSUM GROUP A GENE.**

Kiyoji TANAKA, Naoyuki MIURA, Ichiro SATOKATA, Iwai MIYAMOTO, Hiroshi ASAHINA and Yoshio OKADA. Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka.

Xeroderma pigmentosum group A gene (XPAC) was cloned. The XPAC cDNA encodes single large ORF potentially coding 273 amino acid residues with zinc finger motif. XPAC cDNA was expressed in E. coli by T7 promoter and recombinant XPAC protein was produced and injected into rabbit to elicit polyclonal anti XPAC protein antibody. The immunoprecipitation of crude cell extract with this antibody revealed the 40 and 42 kDa proteins in normal human cells, but not in group A XP cells. Nucleotide sequencing of XPAC gene showed that 16 out of 21 Japanese group A XP patients were homozygous for the G to C transversion at splicing acceptor site of intron 3. Four Japanese group A XP patients were heterozygous for this mutation. The opposite allele in these patients had a nonsense mutation in exon 3 or exon 6. The G to C transversion was not observed in 11 Caucasians and 2 Blacks with group A XP, and in other complementation groups XP patients and normal donors.

B 2

ESTABLISHMENT OF TRANSGENIC MOUSE EXPRESSING HLA-DQw4 MOLECULE. Y.FUKUI, A.KIMURA, M.YASUNAMI, Y.ESAKI, Y. NISHIMURA and T.SASAZUKI. Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka, Japan

Both HLA DR4-DQw4-Dw15 haplotype and DR2-DQw6-Dw12 haplotype are common haplotypes in the Japanese. These haplotypes show reciprocal association with autoimmune diseases such as insulin dependent diabetes mellitus and immune responses to soluble antigens such as streptococcal cell wall and HBs. In order to investigate the function of the HLA DQ molecule in immune regulation, we established transgenic mouse expressing HLA DQw4 molecule (DQw4-B6) in addition to HLA DQw6 trasgenic mouse reported previously. Genomic genes for the α and β chains of the HLA DQw4 molecule were isolated from EB-YT, homozygous for HLA Dw15 haplotype. An 10kb XbaI fragment from the DQw4A gene and a 20 kb kPNI fragment from the DQw4B gene were injected into fertilized eggs from C57BL/6 mice (B6). One or two copies of DQw4A and DQw4B genes were integrated and transmitted appropriately in one strain. The expression of HLA DQw4 molecule on spleen cells was low upon the isolation but it was increased after a culture in medium for 24 hours. Moreover, the expression of HLA DQw4 moelcule was induced with 50U/ml murine recombinant IL-4 for 24 hurs. Both DQw4-B6 and B6 produced an anti DR4 antibody by an immunization with EB-YT cells. B6 mouse also produced an anti DQw4 antibody, but DQw4-B6 did not, indicating that the DQw4-B6 acquired a tolerance to DQw4-molecule. The HLA DQw4 transgenic mouse will serve a valuable material to study the function of HLA DQ molecule in immunoregulation.

B 3

RECOGNITION OF MHC CLASS II GENE PRODUCTS IN THE CONTEXT OF MHC CLASS I MOLECULE BY CYTOTOXIC T LYMPHOCYTES. Takeshi INAMITSU, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept.Genet., Med.Inst.Bioreg., Kyushu Univ., Fukuoka, Japan)

We have reported a line of transgenic mice expressing HLA-DQw6 molecules, which aquired tolerance to HLA-DQw6 gene products and immune responsiveness to streptococcal antigens restricted by DQw6 molecules. In this study, we analyzed the role of DQw6 molecules in the induction of MLR by using HLA-DQw6 transgenic C57BL/6 mice(DQw6-B6). Both CD4⁺ and CD8⁺ long-term T lymphocyte cell lines were generated from lymph node cells of C57BL/6 mice (B6) stimulated in vitro by irradiated spleen cells from DQw6-B6. The CD4⁺ T cell line proliferated in response to L cells transfected with DQw6 genes and these responses were completely inhibited by either anti-DQ or anti-L3T4 mAbs. The CD8⁺ T cell line lysed splenic LPS blast cells from DQw6-B6 spleen cells but not from B6. The CD8⁺ T cell line also exhibited a cytotoxicity to splenic LPS blast cells from backcross progenies between DQw6 positive DQw6-B6 x DBA/1 F1 and DBA/1 only when target cell expressed both DQw6 and H-2^b, indicating that recognition of HLA DQw6 by the CD8⁺ cytotoxic T cell line was restricted by H-2^b. The cytotoxic activity was inhibited by either anti-Lyt2 or anti-H-2D^b mAb but not by anti-HLA-DQ nor anti-H-2K^b mAbs. Thus, we conclude that the HLA-DQw6 transgene products expressed in DQw6-B6 induce xenogenic MLR in both CD4⁺ and CD8⁺ T cells. The CD4⁺ T cells recognize DQw6 molecule itself, whereas the CD8⁺ cytotoxic T cells recognize DQw6 molecule in the context of H-2D^b.

B 4

POLYMORPHISMS IN HLA CLASS II GENES. Rui Ping DONG, Akinori KIMURA, Ken HATAE, Satoru SENJU, Hirofumi NISHI, and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ. Fukuoka, Japan)

HLA class II molecules (HLA-DR, DQ and DP) are highly polymorphic glycoproteins encoded for by a multigene family in HLA region on short arm of chromosome 6. These molecules play a key role in immunorecognition of natural antigens and may control the susceptibility to several autoimmune diseases. Genetic variations in the immune responsiveness and the disease susceptibility may be determined through the polymorphisms in the HLA class II genes. We have analysed the polymorphisms in exon 2 from the class II genes by PCR and dot-blot hybridization with SSO probes (62 SSOs for DRB gene, 24 for DQB, 25 for DPB, 19 for DQA, and 4 for DPA). To define the DRB alleles, locus-specific and allele-specific PCR methods have been developed. We analysed the HLA class II genes from 238 healthy Japanese individuals and found that about 10% of the population had new alleles of DRB1 genes (six new alleles) or DQB1 genes (one new allele). Moreover, several DRB-DQA-DQB haplotypes which seemed to be specific to Japanese population were defined. These analyses give a genetic basis for anthropological study and for disease study in Japanese.

B 5

POLYMORPHISM IN THE PROMOTOR REGION FROM HLA-DQA GENE. Akinori KIMURA and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka, Japan)

HLA class II genes are one of the most polymorphic and best characterized genes in human. The polymorphisms are mainly found in their exon 2. We have determined the nucleotide sequences of the 5' flanking region (-262 to +20) of the DQA1 gene from 22 cell lines homozygous for HLA. Nine variations were found in this region. In all of the nine alleles, point mutations were found in X and Y boxes that are important for the regulation of the class II genes. These mutations well explained that the expression of DQA gene was usually lower than the other class II genes. Moreover, dot blot hybridization study with SSOs corresponding to the highly variable region (-220 to -190) was performed on the DQA1 genes from 85 cell lines homozygous for HLA and 141 unrelated Japanese individuals with known HLA genotypes. The variations in the 5' flanking region of DQA1 gene were found to be closely associated with specific DRB haplotypes but not with specific exon 2 of the DQA1 gene. These analyses showed that the gene organization of the HLA class II region was well conserved as haplotypes, while the exon 2 of the class II genes was highly variable to be generated by genetic exchange events between alleles.

B 6**POLYMORPHISM IN CYTOPLASMIC DOMAIN OF HLA-DQ β CHAIN.**

Satoru SENJU, Akinori KIMURA, Hideyuki YOSHIZUMI, Nobuhiro KAMIKAWAJI, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka, Japan)

As for the function of the HLA class II molecules, it is well known that the HLA-DR molecules play a key role in immune recognition of foreign antigens. On the other hand, previous studies have suggested that the HLA-DQ molecules were involved in the immune suppression. One of the characteristic features of the HLA-DQ genes is the variation at the splicing junction of the exon 5 from DQB gene. Two forms of the junctional sequence allow long or short cytoplasmic tail of DQ β chain. Only the DQB1*0503 and 0601 alleles utilize the exon 5 and encode for a long cytoplasmic tail. To demonstrate a function of the cytoplasmic tail of DQ β chain, we have constructed DQB1*0601 short gene by connecting the DQB1*0601 gene to the DQB1*0401 gene which lack the exon 5. DQB1*0601 or DQB1*0601 short genes were introduced into murine L cells along with DQA1*0103 gene to obtain transfectants expressing wild type or short cytoplasmic form of DQw6 molecules. Both type of the DQw6 molecules could be served as restriction molecule for the SCW specific DQw6 restricted Tcell line. These results demonstrated that the cytoplasmic tail of the DQ molecule may be dispensable for antigen presentation.

B 7**ABH SECRETION IN RELATION TO DIETARY LECTINS AS A MAJOR FACTOR IN NATURAL SELECTION**

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The paper highlights the adaptive significance of secreted blood group antigens in relation to lectin activity in diet. A selection-relaxation hypothesis has been developed to account for widely varying frequencies of the secretor gene in human population. Supportive evidence is derived from serological studies in non-human primates. Selective processes operating in favour and those operating against the nonsecretors are discussed emphasizing the role of dietary lectins as a major factor in the establishment of ABH polymorphism in man.

B 8

FERTILITY, MORTALITY AND ANTHROPOMETRIC DIFFERENCES AMONG SICKLE HEMOGLOBIN CARRIERS AND THEIR NORMAL SIBLINGS

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The Konda Dora and Bagatha tribes from Andhra Pradesh, India are screened for hemoglobin by using cellulose acetate gel electrophoresis. The fertility performance, child mortality and anthropometric measurements are recorded for sickle cell hemoglobin carriers and their sex matched siblings as control sample. Relatively higher fertility and mortality are noticed among the families with one of the partners carrying sickle hemoglobin compared to their normal sibling families. Clear variation is observed for several body dimensions between sickle carriers and controls. The results are presented after applying relevant statistical analysis.

B 9

IDENTIFICATION OF AMINO ACID RESIDUES UNIQUE TO HLA-DRB1 GENES CONTROLLING SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS

Kuniyoshi TSUCHIYA¹, Masakazu KONDO², Akinori KIMURA¹, Yasuharu NISHIMURA¹, and Takehiko SASAZUKI¹ (¹Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., ²Dept. Orthop., Fukuoka-chuo National Hospital, Fukuoka, Japan)

We performed serological and DNA typing of HLA alleles in 188 Japanese patients with rheumatoid arthritis(RA) to identify precise susceptible alleles to RA. Serological typing confirmed the previous reports that HLA-DR4, DRw53, DQw4 were increased in RA patient group compared with 213 healthy individuals. In DR4 negative patients with RA, increased frequency of DR1 and DR9 was observed, indicating that DR1 and DR9 seemed to be other susceptible alleles for developing RA. In Japanese, serologically defined DR4 is further divided into at least three groups Dw4, Dw15, and DKT2 by mixed lymphocyte reaction. To characterize further DRB genes associated with RA, we performed DNA typing of DRB1 genes in 103 DR4 positive patients by testing hybridization between sequence specific oligonucleotide probes and DRB genes amplified in vitro with polymerase chain reaction. DRB1*0405(Dw15) and DRB1*0401(Dw4) were increased. In contrast, DRB1*0406(DKT2) and DRB1*4.9, which has the same sequence as DRB1*0405, except for only one substitution of valine instead of glycine at amino acid residue 86, were not increased in the patients. The comparative analysis of HLA-DRB1 genes between susceptible and non-susceptible alleles to RA revealed the importance of glycine at position 86 in association with non-acidic amino acid (arginine or glutamine) at position 70 for the susceptibility to RA.

B 10

Suppressive effect of HLA-DQw6 gene on collagen induced arthritis in mice:

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In the patients with rheumatoid arthritis (RA), the frequency of HLA-DR4-DQw4 haplotype was increased, on the other hand the frequency of HLA-DQw6 was decreased significantly, suggesting that HLA-DQw6 may control resistance to RA. We investigated the effect of HLA-DQw6 gene on collagen induced arthritis (CIA) in mice, using the F1 progenies with or without DQw6 genes between DBA/1J mice and hemizygous HLA-DQw6 transgenic C57BL/6 (DQw6-B6) mice. Mice were immunized with bovine type II collagen (C II). The incidence of arthritis was observed and the levels of IgG specific to C II were determined on ELISA every week until 10 weeks after the first immunization. The incidence of arthritis in DQw6(+)-F1 was 42.9 % , and that in DQw6(-)-F1 was 80.0 %. The level of IgG specific to C II of DQw6(+)-F1 was 218.6 ± 46.4 (mean $\pm$ S.E.) $\mu\text{g/ml}$, and that of DQw6(-)-F1 was 459.2 ± 77.0 $\mu\text{g/ml}$ on the 5th week, ($P < 0.05$). Thus HLA-DQw6 gene suppressed the development of CIA and the production of IgG specific to type II collagen.

B 12

MOLECULAR ANALYSIS OF PSORIATIC ARTHRITIS. Masahiko MUTO¹, Takahisa OHTA¹, Shinya MATSUURA², Tadashi KAJI², Chidori ASAGAMI¹ (¹Dept. Dermatol., Yamaguchi Univ. Sch. Med., Ube, ²Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Psoriatic arthritis (PA) is a rare, autosomal dominant disease. We previously reported that PA is associated with an HLA-A2-Bw46-DRw8 haplotype (primary associated antigen is HLA-A2) based on the result of a population survey on 19 patients. In addition, the susceptibility gene in linkage disequilibrium with the HLA-A2 seems to be inherited in a dominant fashion.

In order to elucidate the HLA-linked PA susceptibility gene, RFLP of exon 2 of HLA-A2 gene was analyzed by the PCR technique. The fragments, amplified with the primers capable of detecting exon 2 of HLA-A, -B and -C genes, were digested with Dra II, Taq I, Bam HI, Sca I and Pvu II. Dra II could distinguish the fragments of HLA-A2 gene from those of HLA-B and -C genes. Further molecular analysis on the susceptibility to PA is now under way.

B 13**MEIOTIC-STAGE-DEPENDENT RADIOSENSITIVITY IN OVARIAN OOCYTES OF THE MAMMAL.**

Hiroyuki TATENO, Yujiroh KAMIGUCHI, Kazuya MIKAMO (Dept. of Biol. Sci., Asahikawa Med. Col., Asahikawa)

Mammalian oocytes change their sensitivity to lethal, clastogenic and teratogenic effects of radiation with their meiotic process. In our previous studies, we found that immature oocytes of neonatal Chinese hamsters were radioresistant at the pachytene stage, but became sharply sensitive during the phase between diplotene and early dictyate, suffering acute oocyte-killing. However, they became resistant again at the resting dictyate stage. Fertility span was shortened dramatically in the irradiated females with 1 Gy X-rays according to their age of irradiation, indicating that reduction in the number of immature oocytes by acute killing was responsible for the short reproductive life. On the other hand, there was no increase of chromosome aberrations in female pronuclei of one-cell embryos derived from the irradiated oocytes. No increase was also observed in the incidences of pre- and postimplantation death and of congenital malformation. These findings suggest that the oocytes which had survived X-ray at the pachytene and resting dictyate stages are highly capable of repairing chromosome damages; thus they are free from late deleterious effects.

B 14**MICRONUCLEUS TEST OF HUMAN SPERM CHROMOSOMAL DAMAGES.**

Yujiroh KAMIGUCHI, Hiroyuki TATENO and Kazuya MIKAMO (Dept. of Biol. Sci., Asahikawa Med. Col., Asahikawa)

A method of micronucleus test to assess the radiation-induced chromosomal damage in human spermatozoa was described. Its efficiency was found to be similar to that of sperm chromosome analysis.

Human spermatozoa were exposed in vitro to 1.1 and 2.1 Gy of ^{137}Cs gamma rays. After interspecific in vitro fertilization of irradiated spermatozoa with zona-free hamster oocytes, a total of 193 monospermic eggs were examined for micronucleus test at 2-cell stage, and a total of 304 male pronuclear chromosome plates were analyzed according to our established method. The incidence of two-cell embryos with micronuclei coincided well with the incidence of spermatozoa with structural chromosome aberrations (51.6 % vs 52.2 % in the 1.1 Gy group and 82.7 % vs 79.3 % in the 2.1 Gy group). A similar correlation was also found between the number of micronuclei per embryo and the number of structural chromosome aberrations per spermatozoon (0.85 vs 0.92 and 1.50 vs 1.51 in the two dose groups, respectively). These results indicate that our micronucleus test is useful as a simple and rapid method for assessing clastogenic effects of various environmental mutagens on human sperm chromosomes.

B 15

ABERRANT EXPRESSION OF HUMAN IL-3 GENE IN A LUNG CANCER CELL LINE. Takeshi OTSUKA¹, Takanori TESHIMA¹, Tsunefumi SHIBUYA¹, Mine HARADA¹, Hironobu SATOH¹, Seiichi OKAMURA², Yoshiyuki NIHO¹ (¹The 1st Dept. Int Med. and ²Cancer Center, Kyushu Univ., Fukuoka)

Both interleukin 3 (IL-3) and granulocyte macrophage colony stimulating factor (GM-CSF) are produced inducibly from activated T cells. Expression of IL-3 gene is remarkable and sometimes relates to tumorigenicity in mouse. The occurrence of IL-3 mRNA in human T cells was far less frequent than that of GM-CSF mRNA, although they locate quite closely on chromosome 5q and encode proteins of very similar biological activities. Expression of these genes were examined in human cancer cell lines. GM-CSF mRNA is produced in several leukemia cell lines, whereas none of them expresses IL-3 gene. In one of lung cancer cell lines termed KMNT, IL-3 gene transcript shorter than that produced from activated T cells was observed. This is the first report about human IL-3 gene expression in solid tumor cell lines.

B 16

ANALYSIS OF DNA ABNORMALITIES IN NEUROBLASTOMAS AND OTHER CHILDHOOD TUMORS. Shin-ichi YOKOTA^{1,2}, Mieko KATO¹, Atsushi AKANE³, Shigeo NAGAFUCHI¹, Masao YAMADA¹ & Yasuo NAKAGOME¹. ¹Dept. Cong. Abn. Res., Natl. Children's Med. Res. Ctr, ²Dept. Clin. Genet., Kyorin Univ. & ³Dept. Legal Med., Shimane Med. Univ.

We have analyzed DNA samples isolated from various childhood tumors and have reported on N-myc amplification, novel RFLPs in the human N-myc gene and loss of heterozygosity at chromosome 1p in neuroblastomas. Recent progresses in our laboratory in relation to these topics will be reported in the meeting. We reported in 1986 (Mitani et al. Jpn. J. Cancer Res. 77:1062-1065) a case of rhabdomyosarcoma which had the amplified N-myc gene. We have succeeded to establish a cell line from a metastasized tumor of the patient and confirmed that the cells had the amplified N-myc gene and was diagnosed as a rhabdomyosarcoma. Beside of amplification, an abnormal DNA fragment was detected in the original tumor when hybridized with a DNA fragment carrying the 1st exon of the N-myc gene. It was caused for by DNA rearrangement occurred in the upstream region of the N-myc gene. We are in progress to clone the rearranged DNA using the inverted PCR technique.

B 17

p53 GENE MUTATIONS IN FAMILIAL POLYPOSIS COLI. Senji SHIRASAWA, Kazunori URABE, Yuchio YANAGAWA, Koji TOSHITANI, and Takehiko SASAZUKI (Dept.Genet.,Med.Inst.Bioreg.,Kyushu Univ., Fukuoka)

The p53 gene has been elucidated as a tumor suppressor gene and inactivation of the p53 gene caused by deletion or point mutations has been hypothesized to make a role in the development of many human malignancies. In colorectal carcinomas with allelic deletion of the p53 gene, the remaining p53 gene was mutated with considerable frequency. We attempted to determine whether mutations in the p53 gene also occur in tumors from familial polyposis coli (FPC) patients and what stage of tumorigenesis the mutations occur. We established the combined method of polymerase chain reaction and denaturing gradient gel electrophoresis, which could make it easy to detect the mutations in the hot spots of the p53 gene. Using this combined method, we detected the mutations in three adenomatous polyps (3/45,7%) and one carcinoma (1/15,7%) from FPC patients and three carcinomas (3/15,20%) of sporadic cases. These results suggested that the p53 gene mutations might be involved in formation not only of carcinomas but also of adenomas from FPC patients.

B 18

MOLECULAR ANALYSIS OF tcl-1 LOCUS DERIVED FROM THE BREAKPOINT OF CHROMOSOME TRANSLOCATION IN PATIENTS WITH ATAXIA TELANGIECTASIA. Masaharu ISOBE, Nobuyuki KUROSAWA, Harufumi MUTO, Hideki NAGUMO and Zen-ichi OGITA (Dept.of Patho-biochem., Research Inst. for Oriental Medicine, Toyama Med. & Pharma. Univ.)

Ataxia telangiectasia (AT) is a rare autosomal recessive disorder characterized by hypersensitivity to ionizing radiation, increased chromosome breakage, and high incidence of cancer particularly T-cell malignancies. Nonrandom chromosome translocations such as t(14;14)(q11;q32), and t(7;14)(q35;q32) are often observed in the T-cell leukemia of patients with AT. We have shown that loci for T-cell receptor (TCR) such as T α or T β locus at chromosome 14q11 or 7q35, respectively are often involved in translocations with the third locus, named tcl-1 (T-cell lymphoma, leukemia-1) locus at chromosome 14q32.1.

We now obtained about 100Kb of genomic clones around tcl-1 locus by chromosome walking and found 14q32.1 breakpoints of t(14;14)(q11;q32) of an AT and a non-AT patient have been mapped within 10Kb of each other, indicating that the leukemic disease which develops in AT is probably of the same etiology as that of non-AT patients, albeit occurring greater frequency in the former.

B 19

FREQUENCIES OF PHENOTYPES, GENOTYPES, AND DISEASE-RELATED ALLELES FOR APRT DEFICIENT FAMILIES AMONG JAPANESE. Naoyuki KAMATANI[†], Sanae OHTSUKA[†], Masayuki HAKODA[†], Akio MIMORI[‡] and Yuji HIDAKA[‡]. [†]Institute of Rheumatology Tokyo Women's Medical College, and [‡]Department of Internal Medicine University of Michigan

About 79% of all the Japanese families with APRT deficiency possess a mutant allele designated *APRT*J* having a common base substitution probably derived from a single mutation generated many years ago (130,000 years ago as calculated from the linkage analysis). All non-*APRT*J* alleles (*APRT*Q0*) code for complete enzyme deficiency. We analyzed samples from 45 Japanese families (90 alleles) with APRT deficiency to determine phenotypes, genotypes and alleles. Phenotypically, 34 (76%) of the families were of type II (partial deficiency), while the remaining 11 (24%) were of type I (complete deficiency). Of the 34 type II patients, 27 had a genotype of *APRT*J/APRT*J*, while the remaining 7 had a genotype of *APRT*J/APRT*Q0*. All type I families had a genotype of *APRT*Q0/APRT*Q0*. Among the 90 disease-related alleles tested, 61 (68%) had a mutation specific to *APRT*J*, while all 29 (26%) *APRT*Q0* alleles did not. A common base substitution found in some *APRT*Q0* alleles by Mimori et al. and Sahota et al. was detected in 23 (79%) of all the 29 *APRT*Q0* alleles. All of the 23 *APRT*Q0* alleles with the common mutation were linked to the same haplotype concerning the TaqI and SphI RFLP sites present within and near the APRT gene, and considered to be derived from a single mutation. Thus, a single mutation (*APRT*J*) explains 68% of all the disease-related APRT alleles, and two mutations (*APRT*J* and common *APRT*Q0*) explains 93% of all the disease-related alleles among Japanese.

B 20

HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE DEFICIENCY: A MUTATION COMMON TO SEVERAL NULL TYPE ALLELES (*APRT*Q0*) AMONG JAPANESE. Akio MIMORI[†], Yuji HIDAKA[†], Veda C. WU[†], Susan A. TARLE[†], Naoyuki KAMATANI[†], William N. KELLEY[†], and Thomas D. PALELLA[†]
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Adenine phosphoribosyltransferase (APRT) deficiency is a relatively common genetic disorder among Japanese. Patients have been classified as type I (complete deficiency) and type II (partial deficiency), and the mutant alleles have been classified as *APRT*J* (partial deficiency) and *APRT*Q0* (complete deficiency). A single common base change has been identified for all *APRT*J* alleles present in all separate type II patients (Hidaka et al). The present report describes a common mutation present in several *APRT*Q0* alleles among Japanese. DNA was extracted from lymphoblastoid cell lines from 4 unrelated Japanese subjects (3 homozygotes and a heterozygote) all related to the type I deficiency. The entire APRT gene was amplified by PCR and directly sequenced for all the four cell lines. The nucleotide substitutions common to all the seven affected alleles were identified in exon 3; a G to A change at position 1453 (Trp⁹⁸ to a stop codon) and a C to T change at 1456 (degeneration for Ala⁹⁹), and the former change (nonsense mutation) was considered to be disease-causing. These point mutations were demonstrated on PCR amplified cDNA as well. Additionally, two common base substitutions were also identified on the mutant alleles from two homozygotes examined in intron 2 and in intron 3. Thus, a common mutation is present not only for *APRT*J* alleles but also for *APRT*Q0* alleles among Japanese.

B 21

ASSOCIATION OF A MUTANT ALLELE OF CYTOCHROME P450 DB1 GENE WITH PARKINSON'S DISEASE; A MOLECULAR APPROACH TO PARKINSON'S DISEASE
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Since the factors affecting the neurotoxic effects of MPTP may be related to cause of idiopathic Parkinson's disease (PD), and MPTP is converted to inactive metabolites by cytochrome P450 db1 (P4502D1), we studied restriction fragment length polymorphisms (RFLPs) of P4502D1 gene in 22 unrelated healthy subjects and 47 patients with PD. Xba I and Bam H I defined four alleles and two alleles in normal population and three alleles and two alleles in PD population. Gene frequencies of Xba I alleles were significantly different in two populations. However, these of Bam H I alleles were equal. Then, the relative risk of PD in 44 kb homozygotes of Xba I polymorphism compared to 29 kb homozygotes, and difference was significant at 5 level. These data suggest that 44 kb Xba I allele may be one of the susceptible genetic factors to acquire PD in human.

B 22

MOLECULAR ANALYSIS OF VITAMIN D RECEPTOR IN PATIENTS WITH VITAMIN D-DEPENDENT RICKETS TYPE II. Takahiko SAIJO¹, Michinori ITO¹, Eiji TAKEDA¹, A.H.Mahbubul HUQ¹, Etsuo NAITO¹, Yasuhiro KURODA¹ and John Wesley Pike². 1: Department of Pediatrics, School of Medicine, University of Tokushima, Kuramoto 2, Tokushima 770 2: Department of Cell Biology and Pediatrics, Baylor College of Medicine, USA

Vitamin D-dependent rickets type II is a hereditary disease caused by the various defects of vitamin D receptor (VDR). Patients are characterized by early onset of rickets associated with alopecia. Five patients, two siblings and three isolated, were studied for abnormalities in VDR and its gene. The cytosol receptors from the skin fibroblast of the patients showed normal binding of 1,25(OH)₂D₃, but nuclear uptake of [³H]1,25(OH)₂D₃ was impaired. In immunoblot analysis with a monoclonal antibody to VDR, the receptors from the patients migrated at the molecular size of 52kDa, which was indistinguishable from the control subjects. Genomic DNAs from the patients were digested by EcoRI, PstI, TaqI, BamHI and MspI and subjected to Southern blot analysis using human VDR cDNA as a probe. No RFLPs were found in the patients as well as in normal controls. These results suggest that the abnormality of VDR gene may be a more subtle mutation, such as a point mutation, in the region encoding DNA binding domain or nuclear localization domain.

B 23

MOLECULAR GENETIC ANALYSIS FOR THE DYSTROPHIN GENE IN DUCHENNE-TYPE OR BECKER-TYPE MUSCULAR DYSTROPHY IN JAPANESE PATIENTS. Mieko KODAIRA, Keiko HIYAMA, Chiyoko SATOH (Dept. of Genet., RERF, Hiroshima), and Hitoshi KAMEO, Takenori KARAKAWA (Hara Byoin National Sanatorium)

We have studied structural changes at the DNA level in the dystrophin gene of 57 patients (including 10 pairs of siblings) diagnosed at the Hara Byoin National Sanatorium (Hiroshima) with Duchenne or Becker muscular dystrophy (DMD or BMD). DNA from patients was digested with HindIII or SfiI, separated by conventional gel electrophoresis (CGE) or CHEF, and analyzed by Southern blotting with probes covering all regions of dystrophin cDNA. Deletions were detected in 33 cases (58%) and partial duplications were detected in seven cases (12%). Deletions in carriers and partial duplications are sometimes difficult to detect by CGE. We could clearly detect such variations by additional CHEF analysis. Among seven cases of partial duplication, four (57%) were located in the region of cDMD9-10. Though two deletion hot spots are reported to be in region 8 and region 1-2a, they distributed outside of these regions.

B 24

ANALYSIS OF HLA-C GENE IN JAPANESE PATIENTS WITH PSORIASIS VULGARIS BY POLYMERASE CHAIN REACTION.
S. AKASAKI*, H. NAKAGAWA*, A. ASAHIAN*, S. KUWATA**, K. TOKUNAGA**, Y. ISHIBASHI*, and T. JUJI**, Department of Dermatology* and Blood Transfusion Service**, Faculty of Medicine, University of Tokyo, Tokyo, Japan

Psoriasis is an intractable chronic cutaneous disorder affecting up to 4% of USA white population, about 0.05-0.1% of Japanese population. Although the exact mechanism of psoriasis is still unknown, genetic factors are implicated in its etiology. While its genetic mode is not yet fully understood, certain HLA antigens, especially HLA-C (CW6), have been reported to be strongly associated with psoriasis despite racial or ethnic origins. We analyzed the relationship between HLA-C gene and psoriasis by the polymerase chain reaction (PCR) method. DNA was isolated from 24 patients and 37 healthy controls. A segment of the Exon 2 region in HLA-C was amplified specifically by using a pair of primers and Taq polymerase. As probes, we used two kinds of synthetic oligonucleotides coding for 70-75 amino acid residues. Our results showed that the probe corresponding to 73Ala had significantly increased in frequency in patients (88% vs 38%, $P < 0.0002$). Our study suggested that this portion on the Cw6, Cw7 and Cx52 molecules could contribute to the disease susceptibility.

B 25**Molecular Basis of Argininemia: Identification of Two Discrete Frame-shift Deletions in the Liver-type Arginase Gene**

Yougo² Haraguchi,¹ Juan M. Aparicio R.,¹ Makoto Yoshino,³ Masataka Mori,² and Ichiro Matsuda¹ (¹Dept. Pediatr., ²Inst. Med. Genet., Kumamoto Univ.; ³Dept. Pediatr., Kurume Univ.)

Argininemia results from a deficiency of arginase, the last enzyme of the urea cycle in the liver. We examined the molecular basis for argininemia by constructing a genomic library followed by cloning and DNA sequencing. Discrete mutations were found on two alleles from the patient, a product of a nonconsanguineous marriage. There was a four base deletion at protein-coding region 262-265 or 263-266 in exon 3 that would lead to a reading-frame shift after amino acid residue 87 and make a new stop codon at residue 132. The other was a one base deletion at 77 or 78 in exon 2 that would lead to a reading-frame shift after residue 26 and make a stop codon at residue 31. For confirmation, genomic DNAs from the patient and from her parents were amplified by PCR method. The patient was shown to be a compound heterozygote, inheriting an allele with a four base deletion from the father and the other allele with a one base deletion from the mother. DNA analysis of other patients are underway.

B 26**MOLECULAR BASIS OF PROLIDASE DEFICIENCY:
EXPRESSION OF ABNORMAL ENZYMES IN CULTURED CELLS**

Akito Tanoue, Fumio Endo, Ichiro Matsuda
Dept. Pediatr of Kumamoto University

Prolidase deficiency is an autosomal recessive disorder characterized by mental retardation and various skin lesions. We identified a point mutation of a G to A substitution at position 826 in exon 12 in cells from patients with CRM positive prolidase deficiency, and deletion mutation of exon 14 in cells from Japanese female siblings with CRM negative phenotype. An expression plasmid clone containing a normal human prolidase cDNA (pEPD-W), mutant cDNA with a G to A substitution (pEPD-M) or mutant cDNA with a 192 bp deletion of exon 14 (pEPD-K) were prepared, transfected, and tested for expression in cultured cells. Active human enzyme was detected in cells transfected with pEPD-W, but not in those transfected with pEPD-M and pEPD-K. The cells transfected with pEPD-K carried the immunological protein with a smaller size (50 KD). These observations were relevant when considering the structure-function relationship of this unique peptidase.

B 27

MOLECULAR STUDY OF MEDIUM-CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY Yoichi MATSUBARA¹, Kuniaki NARISAWA¹, Shigeaki MIYABAYASHI², Keiya TADA², and Paul M. COATES³ (Dept. of ¹Biochemical Genetics and ²Pediatrics, Tohoku Univ. Sch. of Medicine, Sendai 980, JAPAN ; ³Dept. of Pediatrics, The Children's Hosp. of Philadelphia, Philadelphia, U.S.A.)

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is one of the most common recessively inherited metabolic diseases in man. It has been known to cause sudden infant death and Reye-like syndrome among children. We have studied fibroblast cultures collected from three patients with MCAD deficiency and identified an A(985) to G(985) nucleotide replacement in all cultures. This mutation resulted in the substitution of lysine(329) by glutamic acid(329). The same mutation was found in 12 additional patients in homozygous form, indicating the remarkably high frequency of this point mutation. We have developed a simple 'modified' polymerase chain reaction method for detecting the mutation. This method utilizes dried blood spots on Guthrie cards and could be used to diagnose this disorder.

B 28

cDNA - MEDIATED EXPRESSION OF HUMAN ORNITHINE AMINOTRANSFERASE Yoshihiro Hotta* and George Inana**, * Dept. of Ophthalmol., Juntendo Univ., Tokyo, Japan, ** Bascom Palmer Eye Institute, Miami, U.S.A.

Gyrate atrophy is a hereditary chorioretinal degenerative disease caused by a deficiency of the mitochondrial enzyme, ornithine aminotransferase (OAT). A gene therapy for this disease may be possible if a cloned OAT gene or cDNA can be introduced into and expressed in the defective tissue. We investigated the expression of a cloned human OAT cDNA after its introduction into mammalian cells by DNA - mediated transfer (transfection). We have previously isolated a cDNA clone for the human OAT(pHOAT) that contains the entire coding sequence. This clone was inserted into an eukaryotic expression vector(pcD) which contains the SV40 early promoter. NIH3T3 cells and OAT(-) Chinese hamster ovary(CHO) cells were used for the transfection. Incorporation of pcDHOAT was detected in both cell types with expression of the human OAT mRNAs and active OAT enzyme proteins confirmed by Southern, northern, and western analysis and OAT enzyme activity assay.

B 29

A PROPOSAL OF GENOMIC IMPRINTING MODEL IN ANGELMAN SYNDROME. Kenji NARITOMI (1,2), Tatsuro KONDOH (2), Michio YASUNAMI (2) and Akira YOSHIDA. (1)Dept., Pediatr., Univ. Ryukyus Sch. Med, Okinawa, Japan (2) Dept. Biochem. Genet., Beckman Res. Inst. City of Hope, Duarte, CA, USA

The maternal genomic imprinting hypothesis in Prader-Willi syndrome (PWS) is inferred from preferential deletions of paternal 15q and DNA alleles, as well as maternal disomy (Nicholls et al. 1989). In contrast, paternal imprinting has been suggested in Angelman syndrome (AS) (Williams et al. 1990).

However, we concluded that maternal genomic imprinting of the AS gene should be more likely as a result of comparisons among previous data concerning to 15q11.2 genomic DNA-derived cDNA mapping, mouse genomic imprinting map, human/mouse comparative mapping, and molecular deletions in PWS and AS. In the hypothesis, preferential maternal deletions in AS is explained by presuming a suppressor gene against the AS gene. In the model, AS phenotype is expected by overexpression of the AS gene due to inactivation of the paternal suppressor gene. In contrast, paternal deletions induce underexpression which results in non-AS or normal phenotypes.

B 30

MOLECULAR ANALYSIS OF mtDNA DELETIONS IN CHRONIC PROGRESSIVE EXTERNAL OPHTHALMOPLÉGIA (CPEO) AND CORRELATION WITH THE CLINICAL FEATURES Yoshinori TANNO¹, Makoto YONEDA¹, Toyoaki YAMAUCHI¹, Hiroaki MIYAJIMA², Hidetomo TAKAHASHI³, Satoshi HORAI⁴, Takayuki OZAWA⁵, Tadashi MIYATAKE¹, and Shoji TSUJI¹. (Brain Res. Inst., Niigata Univ.¹, Hamamatsu Med. School², Shizuoka Natl. Hosp.³, Natl. Inst. Genet.⁴, Nagoya Univ.⁵.)

Chronic progressive external ophthalmoplegia (CPEO) is a clinical phenotype of a mitochondrial disease, which is characterized by ophthalmoplegia and myopathy. We performed detailed analysis of deletions of mtDNA in 7 patients with CPEO. Using Southern blot analysis and PCR (polymerase chain reaction)-RFLP (restriction fragment length polymorphism), 6 cases showed mixed population of a deleted mtDNA and a normal one. One case had multiple deleted mtDNA, while 5 cases had a simple one. Direct sequencing of PCR products containing the deletion breakpoints revealed that 4 cases had an identical common breakpoint and the other cases had different breakpoints. We did not find any correlation between clinical features and the populations or sites of deleted mtDNA.

B 31

A CASE REPORT OF LONG-ALIVE DMD BROTHERS WITH AN IN-FRAME DELETION OF THE DYSTROPHIN GENE. Natsuki IMOTO¹ Kiichiro MATSUMURA³ Tadao ARINAMI² Kenzo HAMANO¹ Hitoshi TAKITA¹ Hideo HAMAGUCHI² (¹ Dept. Pediatr., ³ Dept. Hum. Genet., Univ. Tsukuba, Tsukuba, ² Dept. Neurol., Shimoshizu Natl. Hosp., Yotsukaido)

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic disorders. Monaco et al.(1988) hypothesized that the reading frame was disrupted by internal deletions in DMD but not in BMD. We experienced DMD brothers with an in-frame deletion of the 5' region of the DMD gene. Using cDMD1-2a as a probe, Southern blot analysis revealed that 4.2-kb HindIII fragment and 2.9-kb BglII fragment were absent in both cases. Since exon 3 of the DMD gene is represented by 4.2-kb HindIII fragment (Darras et al. 1988), it is suggested that 2.9-kb BglII fragment represents exon 3 (Ionasescu et al. 1989). Although wheelchair bound since 12 and 11 years of age respectively, they, 36 and 33 years old, are still alive and have only mild cardiomyopathy. We discussed the correlation between the phenotype and the deletion of exon 3.

B 32

P 蛋白欠損型非ケトーシス型高グリシン血症日本人兄弟例の病因遺伝子解析 呉 繁夫, 高柳 勝, *久米晃啓, 成澤邦明, *多田啓也 (東北大学医学部病態代謝, *同小児科)
GENETIC ANALYSIS OF NONKETOTIC HYPERGLYCINEMIA DEVOID OF P-PROTEIN —JAPANESE SIBLING CASE Shigeo KURE, Masaru TAKAYANAGI, *Akihiro KUME, Kuniaki NARISAWA and *Keiya TADA (Depts of Biochemical Genetics and *Pediatrics, Tohoku University, School of Medicine)

非ケトーシス型高グリシン血症は、常染色体劣性遺伝をとる先天代謝異常症であり、その病因はグリシン開裂系の障害による。グリシン開裂系は複合酵素系であり、P-, H-, T-, 及びL-蛋白と称される4種類の構成酵素より成る。今回私共はP-蛋白欠損型の本症患者の日本人兄弟例の病因遺伝子解析を行った。両者とも生下時より重度の中樞神経障害を有し、その後、精神運動発達の遅延が著明となり弟は4才時に死亡した。患児の両親に血縁関係はなかった。検索はまず、弟の剖検肝より調製したPoly(A⁺) RNAを用いて行い、次の事が判明した。1) 患者肝におけるP-蛋白 mRNA の発現量は正常肝の約1/2量である事、2) 患者P-蛋白 mRNA を cDNA 化し、構造解析を行った結果蛋白翻訳領域に3塩基の欠失を認め、このためフェニルアラニン残基が1つ欠落したP-蛋白をコードしていた。この変異はCos細胞を用いた発現実験からP蛋白活性を完全に失なわせる事が分った。兄弟ともにゲノムDNAは、3塩基欠失アレルとその欠失のないアレルのヘテロ接合体であり、患者肝で発現しているのは3塩基欠失アレルのみであった。また、3塩基欠失アレルは母親由来であり、父親由来のP-蛋白遺伝子は、患者肝で発現していない事から、この父親由来の遺伝子には mRNA を発現出来ない様な別の変異が存在していると考えられた。従って患児らは compound heterozygote と判断された。

B 33

PCR ANALYSIS OF Y CHROMOSOME. Yasuo NAKAGOME, Satoko SEKI, Shigeo NAGAFUCHI, Yutaka NAKAHORI (Dept. Cong. Abn. Res., Nat. Child. Med. Res. Ctr., Tokyo)

PCR detection of different segments of human Y chromosome, along its entire length, was attempted and specificities of individual PCR reactions were evaluated. No part of the Y10, a repeating unit of the DYZ1 family was found to be strictly male specific. As to the non-fluorescent segments, two PCR reactions were found to detect both X and Y loci simultaneously. Both the presence of the DNA and the success of reaction can be monitored by the detection of the X locus and the sex of the sample is determined based on the Y locus, an important feature for forensic or other purposes dealing with small amount or deteriorated samples. A dual amplification system was also developed with which 1/50,000 mosaicism was detectable.

B 34**ACID CARBOXYPEPTIDASE DEFICIENCY IN GALACTOSIALIDOSIS.**

Nobuaki TAKIYAMA^{1,2}, Kohji ITOH¹, Akihiro OSHIMA¹, Yoshiro NAGAO¹, Hitoshi SAKURABA¹, Michel POTIER³, Yoshiyuki SUZUKI¹ (¹Dept. Clin. Genet., Tokyo Metr. Inst. Med. Sci., Tokyo, ²Saiseikai-Utsunomiya Hosp., Utsunomiya, ³St. Justine Hosp., Quebec)

Galactosialidosis is an autosomal recessive genetic disorder caused by a deficiency of "protective protein" which stabilizes lysosomal β -galactosidase and activates lysosomal neuraminidase. Recently human "protective protein" cDNA has been cloned by Galjart et al, and the deduced amino acid sequence was found to possess homology to yeast carboxypeptidase Y and the KEX 1 gene product. In this study acid carboxypeptidase of fibroblasts and/or lymphoblasts derived from various clinical type of galactosialidosis patients was assayed using N-CBZ-L-phenylalanyl-L-leucine as substrate. The result revealed that acid carboxypeptidase activity was deficient in the cells from patients of early infantile and juvenile/adult forms. The enzyme was found to be a serine carboxypeptidase homologous to yeast carboxypeptidase Y, in which histidine and cysteine residues are responsible for the catalytic activity.

B 35

Characterization of the mutation in a male patient with ornithine transcarbamylase deficiency : Toshinobu MATSUURA^{1,2}, Akira HATA^{1,2}, Chiaki SETOYAMA¹, Kazunori SHIMADA¹, Yutaka HASE³, Izumi AKABOSHI² and Ichiro MATSUDA² (¹Department of Biochemistry and ²Department of Pediatrics, Kumamoto University Medical School, Kumamoto. ³Children's Medical Center of Osaka City)

Ornithine transcarbamylase (OTC) deficiency is the most common inborn error of the urea cycle and shows an X-linked inheritance. Deficiency of OTC in hemizygous male patients results in neonatal hyperammonemia, coma, and death in childhood. In this report, we analysed the mutation in OTC gene derived from a male patient with mild OTC deficiency. We sequenced all the exons and exon/intron junctions of the OTC gene after amplifying them by PCR method. We found that the mutant OTC gene carries an A-to-T substitution in a FokI site of exon 6, and also found that this substitution alters aspartic acid at position 196 to valine. To analyze the segregation pattern of the normal and mutant OTC alleles, we conducted the family study by FokI digestion of the amplified DNAs and allele-specific oligonucleotide (ASO) hybridization. We found that his mother is heterozygous for the OTC gene, carrying one normal and one mutant gene.

B 36

DNA ANALYSIS IN A PATIENT WITH AMELOGENESIS IMPERFECTA. Shigeo NAGAFUCHI¹, Yutaka NAKAHORI¹, Takashi TAMURA^{1,2}, Hidefumi TONOKI³, Kenji FUJIEDA³, Tetuo SHIRAKAWA⁴, Yasuo NAKAGOME¹. (¹Dept.Cong.Abn. Res., Nat.Child.Med.Res.Ctr., Tokyo; ²Dept.Clin.Genet., Kyorin Univ. School Hlth.Sci., Tokyo; ³Dept.Pediat., Univ.Hokkaido, Sapporo; ⁴Dept. Pedodont., Univ.Hokkaido, Sapporo)

Amelogenin is the major protein in the developing enamel matrix. And amelogenesis imperfecta is a human genetic disorder affecting the formation of enamel matrix. We cloned human amelogenin gene and determined its nucleotide sequences of the most conserved region. In the present report, we have examined a male patient with amelogenesis imperfecta whether this gene is responsible to this disease.

Two different approaches were employed: First, genomic DNA from the patient was digested with nine different restriction enzymes and analysed by Southern hybridization; Second, nucleotide sequences of the three exons were determined by direct sequencing of genomic DNA amplified by the polymerase chain reaction. With these two methods we couldn't reveal any mutations of human amelogenin gene, so far. Analysis of additional patients is in progress.

B 37

DIAGNOSIS OF HETEROZYGOUS STATES FOR APRT DEFICIENCY BY THE DETECTION OF *IN VIVO* SOMATIC MUTANTS IN BLOOD T CELLS : Masayuki HAKODA¹, Shoko KUROSHIMA¹, Naoto KAMATANI², Naoyuki KAMATANI¹
(¹Institute of Rheumatology, Tokyo Women's Medical College, ²Chikugo City Hospital)

It is usually difficult to make an absolute distinction between normal and heterozygous individuals for autosomal recessive disorders by the measurement of gene product using biochemical procedures. Recently, we have detected mutant T cells deficient in APRT activity at a high frequency (on the order of 10^{-4}) in the peripheral blood of heterozygotes for APRT deficiency but at a very low frequency (on the order of 10^{-9}) in normal individuals. Thus, the existence of APRT-deficient mutant T cells in peripheral blood may be a good indicator of heterozygous states for APRT deficiency. Peripheral blood mononuclear cells (PBMC) were cultured in the presence of interleukin-2 and 2,6-diaminopurine. APRT-deficient T cells could be obtained from all of 15 putative heterozygotes by testing 2×10^6 PBMC from each individual. Such T cells were also detected in 6 out of 11 family members who were not obligate heterozygotes. On the other hand, no mutants were detected from as many as 1.17×10^9 PBMC from 7 normal individuals. We then screened 425 Japanese individuals from 2 residential areas and could successfully find 2 heterozygotes (0.47%). The detection of *in vivo* mutational events in somatic cells may be utilized to identify heterozygotes of other genetic disorders with autosomal recessive inheritance.

B 38

学校検尿で発見され, pseudodominant遺伝形式を示した adenine phosphoribosyl transferase (A P R T) 欠損症の親子例. 石館 武夫, 五十嵐すみ子, 鎌谷 直之, 北里大学小児科, 長津田厚生総合病院, 神奈川県予防医学協会, 東京女子医科大学リウマチ・痛風センター. PSEUDODOMINANT TRANSMISSION OF AN AUTOSOMAL RECESSIVE DISEASE, ADENINE PHOSPHORIBOSYLTRANSFERASE DEFICIENCY: IDENTIFICATION OF THE PROPOSITUS IN THE ROUTINE SCHOOL URINALYSIS PROGRAM. Takeo ISHIDATE, Sumiko IGARASHI, Naoyuki KAMATANI. (Dept. of Ped. Kitasato Univ. Sagami-hara, Nagatsuta Kousei General Hosp. Kanagawa Health Serv. Assoc. Yokohama, Instit. of Rheumatol. Tokyo Women's Medical College, Tokyo)

症例は12歳の女兒。学校検尿で血尿, 蛋白尿, 白血球尿, 細菌尿が発見され, 尿沈渣に2-8 dihydroxyadenine (DHA)らしき結晶を多数認めた。父親はDHA腎結石の既往歴があり家族をT細胞法で調べたところ, 父と発端者はhomozygote, 母と弟はheterozygoteであった。赤血球中のAPRT活性は発端者も父も部分欠損(タイプII)であった。PCRとallele specific oligonucleotide hybridizationを用いた遺伝子診断法にて発端者と父はAPRT*J対立遺伝子のみを, 母と弟はAPRT*J.non-APRT*Jの両方の対立遺伝子を持っていることが示された。従って父と発端者の遺伝子型はAPRT*J/APRT*Jで, 母と弟の遺伝子型はAPRT*1/APRT*Jと結論される。homozygoteとheterozygoteという極めて稀な結婚型のため常染色体劣性遺伝の疾患が表現型からは優性遺伝のような遺伝形式(pseudodominant)を示し, また学校検尿にて本疾患が発見されたのは本邦初例である。

B 39

IDENTIFICATION OF MUTATIONS LEADING TO THE LESCH-NYHAN SYNDROME IN TWO UNRELATED JAPANESE PATIENTS. Shin FUJIMORI, Tetsuo TAGAYA, Noriko YAMAOKA, Naoyuki KAMATANI¹ and Ieo AKAOKA. Second Department of Internal Medicine, University of Teikyo, Tokyo ¹Institute of Rheumatology, Tokyo Women's Medical College, Tokyo

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency is an inborn error of purine metabolism. Complete deficiency of HPRT is associated with Lesch-Nyhan syndrome, an X-linked, genetically lethal, neurological disorder. We have identified single point mutations in two Japanese Lesch-Nyhan subjects by sequencing of the mutant HPRT cDNA amplified using polymerase chain reaction (PCR). One is a C to T change at nucleotide position 151 in patient T.H., which leads to the generation of a nonsense codon (TGA) at amino acid position 51, resulting in absence of catalytic activity in the HPRT protein. The other is a G to A transition at 419 base position in patient Y.Y., which predicts a single amino acid substitution of an aspartic acid for a glycine at position 140. These mutations are not identical to any other previously identified HPRT variants in the world.

B 40

EVIDENCE OF GENETIC HETEROGENEITY IN AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA
S.S.PAPIHA., C.G.INGLEHEARN and S.S.BHATTACHARYA
Department of Human Genetics, Univeristy of Newcastle upon Tyne, England.

Molecular genetic linkage studies have been in progress in five large autosomal dominant retinitis pigmentosa (ADRP) families of British origin. Two of the families display early onset (D-type) of the disease while the remaining have a late onset (R type). Recently tight linkage (0-0, lodscore 14.7) was reported between a DNA marker, C17, from chromosome 3 in a large, early onset, Irish family. In our lab this linkage was not observed in a large late onset family, thereby providing evidence for the genetic heterogeneity in ADRP. However we have been able to confirm the chromosome 3 linkage in one of our early onset families. The gene coding for the rhodopsin also maps very close to the marker C17 which is expressed in the rod photoreceptors and it is known that the photoreceptors degenerate in RP. Recently Dryja and colleagues observed a C A mutation in the 23rd codon of the rhodopsin gene leading to a proline to histidine substitution in 17 out of 148 ADRP patients.

B 41**FAMILY STUDIES OF HYPERCHOLESTEROLEMIC CHILDREN**

Hisako YANAGI¹, Yae SHIMAKURA², Kimiko KOBAYASHI¹, Natsuki IMOTO², Shigeru TSUCHIYA³, and Hideo HAMAGUCHI¹ (¹Dept. Hum. Genet., Inst. Basic Med. Sci., ²Dept. Pediatr., ³Inst. Comm. Med., Univ. Tsukuba)

Two hundred and one children were diagnosed to be hypercholesterolemia from a screening of 2,642 school children in the community near University of Tsukuba, and family studies of these children were performed. More than 60% of the parents participated in the study. Hypercholesterolemia was more frequently in parents of hypercholesterolemic children than controls. Eighteen families with probable autosomal dominant hyperlipidemia were detected. Based on clinical features, we diagnosed three families as familial hypercholesterolemia and six families as familial combined hyperlipidemia. The other nine families were autosomal dominant hypercholesterolemia of unknown etiology. Familial defective apoB-100 were not detected in the nine families by using PCR and allele-specific oligonucleotide probes method. These results indicate that genetic factors are important as cause of hypercholesterolemia of school children and that autosomal dominant hypercholesterolemia of unknown etiology is more frequent than expectation.

B 42**A NONSENSE MUTATION DETECTED IN THE APOLIPOPROTEIN A-I GENE IN A PATIENT WITH APOLIPOPROTEIN A-I DEFICIENCY,**

Tomoyuki MATSUNAGA¹, Yoshikazu HIASA², Hisako YANAGI¹, Kimiko KOBAYASHI¹, Takashi OBARA³, Hideo HAMAGUCHI¹ (¹ Univ. of Tsukuba, Ibaraki, ² Komatsujima Red Cross Hosp., Tokushima, ³ Tsukuba Research Laboratory, Ibaraki)

The apolipoprotein A-I (apo A-I) gene from a female patient with apo A-I deficiency and premature coronary heart disease has been analyzed. Sequencing after amplification by the PCR revealed the presence of a codon 84 nonsense mutation of exon 4 and a codon 37 missense mutation of exon 3 in the apo A-I gene. The data on dot blot hybridization with allele-specific oligonucleotide probes indicated that she is homozygous for the apo A-I gene with the two mutations. Her parents were first cousin marriage. The heterozygote of the codon 37 missense mutation of the apo A-I gene was observed in six out of 60 healthy controls and the six had normal level of apo A-I, suggesting that the missense mutation is polymorphic and not associated with the apo A-I deficiency. These findings indicate that homozygosity of the apo A-I gene with the codon 84 nonsense mutation causes apo A-I deficiency.

B 43**DIAGNOSIS AND PRENATAL DIAGNOSIS OF LYSOSOMAL STORAGE DISEASES
IN NORTH CHINA**

Wilson H. Y. LO, Hui-ping SHI and Nian-hu SUN

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The lysosomal storage diseases rank second (only next to PKU) among the inherited metabolic diseases seen in the PUMC Pediatric Genetic Clinic over the past 6 years. A total of 47 cases of mucopolysaccharidoses, sphingolipidoses or mucolipidoses were diagnosed according to clinical manifestations and specific enzyme assays. Mid-trimester prenatal diagnoses were performed in 11 pregnancies at risk and the diagnoses were confirmed in the abortuses or newborns.

The results were discussed.

B 44**Prenatal Diagnostic Program in China**

Nianhu Sun, Chinese Association of Human Genetics Peiking Union
College Hospital, Beijing, China

In China, prenatal diagnostic program becomes more important due to the "one family one child" policy of the government. Therefore there is a great demand for the doctors to prevent the delivery of congenital and hereditary diseased baby. The prevalence of congenital disease was quite high. It was 13 % in the data of birth defect screeing program of China in between 1986,10-1987,9. The NTD was the major kind birth defect in the northern eastern district of China. The chromosomal aherration rate of chromosomal screening program in 3415 cord blood from Honan in 1979-1982 was found to be 0.37% the mental retarded children in Beijing was 1 % . The prenatal diagnostic program was started in 1977 in PUMC Hospital and 3000 cases of amniocentesis and assay of alphafetoprotein of amniotic fluid was done 1500 cases of amniotic cell culture was done, the CHR aberration rate was 1.5-3%.The NTD rate detected was 6%. Other CNS, DNA technology, was examination was also discussed.

B 45

COMPARATIVE STUDY ON THE INFANTILE AND ADULT FORM OF PHOSPHOFRUCTOKINASE DEFICIENCY ; M. OWADA, M. ONO, Y. YOSHIDA, T. KITAGAWA, K. SARUKI* and T. KOBAYASHI*; Dept. of Pediatr., Nihon Univ., Sch. of Med., 1-8, Kandasurugadai, Chiyoda-ku, Tokyo, Dept. of Urology, Gunma Univ., Sch. of Med., 3-39, Shouwa-machi, Maebashi, Gunma*

Genetic deficiency of phosphofructokinase (PFK), is known as one type of muscular glycogen storage disease (GSD VII). Clinical features of GSD VII usually start from the 2nd decade. However, recently several reports indicated that PFK deficiency is found among patients with congenital myopathy. We have experienced both types of PFK deficiency in 2 families. In one family, muscular pain and weakness were seen from teenage period in two siblings, and finally, the elder brother got acute renal failure after playing baseball. He received peritoneal dialysis. Phosphorylase as well as PFK activity were measured using skeletal muscle, and PFK was decreased by about 5% of controls. In the other family, a floppy newborn baby was examined. Serum CPK was markedly elevated, and clinical features were similar to congenital myopathy of Fukuyama. However, electron microscopical analysis revealed numerous glycogen granules and PFK activity was reduced to 1/10 of control subjects. In both cases, total erythrocyte PFK activity did not differ from control values. To clarify the metabolic basis on clinical differences of both types, immunochemical investigations are carrying out.

B 46

MAPLE SYRUP URINE DISEASE CAUSED BY A PARTIAL DELETION IN THE mRNA OF THE INNER E2 CORE DOMAIN OF BRANCHED CHAIN α -KETOACID DEHYDROGENASE COMPLEX

Hiroshi Mitsubuchi, Yoshitaka Nobukuni, Izumi Akaboshi, Yasuhiro Indo, Fumio Endo, Ichiro Matsuda (Dept. Pediatr., Kumamoto Univ. Kumamoto)

We have studied the molecular bases of maple syrup urine disease by analyzing the activity, subunit structure and mRNA sequence of the affected enzymes. The branched chain α -ketoacid dehydrogenase (BCKDH) activity in the patient was 4.2-4.5 % of control level. Immunoblot analysis revealed that the E2 subunit of BCKDH (Mr. 52,000) was absent and another protein band with a Mr. of 49,000 was present. We amplified the cDNA of the E2-subunit obtained from the patient's cell, using the polymerase chain reaction method, then sequenced the amplified cDNA, in which a 84 bp deletion was identified. The consanguineous parents and a sister had two species of mRNA; the one corresponding to the normal E2 subunit and the other with a 84 bp deletion, whereas findings in a brother were normal. The molecular size of the translation products, as deduced from the abnormal mRNA sequence was compatible with an abnormal protein band (Mr. 49,000) detected in the patient's cells by immunoblot analysis.

B 47

A CASE REPORT OF ADULT HYPOPHOSPHATASIA. Fujio TAKEUCHI¹, Sachiko TOYODA², Sadayoshi YOSHINOYA^{1,2}, Akiyuki OKUBO², Koji ITO¹. ¹Depart. of Med. & Physical Ther., ²Depart. of Lab. Med., Univ. of Tokyo

Adult hypophosphatasia is a rare genetic disease characterized by low serum alkaline phosphatase (ALP) and osteomalacia. We report a case of man aged 53 years old who complained shoulder stiffness and low back pain, and had a history of early loss of secondary tooth. He showed low serum ALP activity, 28 U/l (normal value 66-220), decreased neutrophil ALP (NAP) score, 16% of control, increased serum VB6, 165.1 ng/ml (3.6-18) and normal level of serum Ca, P, Mg, parathyroid hormone and calcitonin. He showed normal urinary and serum phosphoethanolamine. Serum 25-OH VD, 24,25-(OH)₂VD and 1 α ,25-(OH)₂VD in December was 25.2 ng/ml, 0.59 ng/ml and 91.0 pg/ml respectively (14.8 $\pm$ 5.8, 1.08 $\pm$ 0.26 and 59.7 $\pm$ 14.1 normal value for reference). Considering fluctuations of VD along with seasons, VD values were thought to be within normal range. Bone X-ray examination indicate calcification of tendon and cervical mal-alignment. ALP isozyme showed almost ALP_{2,3} and γ GTP isozyme showed α 1 62%, α 1+ α 2 38%. Isozyme patterns of amylase, creatine phosphokinase, lactic dehydrogenase indicated normal pattern. One of his two sons aged 16 years old showed serum ALP, 122, NAP Score, 63% of control and slightly increased serum VB6, 26.6. His ALP is relatively low for his age, however, we couldn't make a diagnosis at the present time. The frequency of subjects showing decreased ALP activity in Tokyo University Hospital was 5/30715 (0.016% ALP $\leq$ 33).

B 48

HB FUKUTOMI [α 126(H4)ASP $\rightarrow$ VAL]: A NEW HEMOGLOBIN VARIANT WITH HIGH OXYGEN AFFINITY. Kazuo HIDAKA, Iwao IUCHI (Dept. Biochem., Kawasaki Med. Sch., Kurashiki) and Kyosuke YAGUCHI (Dept. Med., Kawasaki Hosp., Okayama)

Hb Fukutomi [α 126(H4)Asp $\rightarrow$ Val], a new Hb variant with high oxygen affinity, log P₅₀=0.201, pH 7.0(normal:1.087), was detected from a 35 yr-old male with no sibling and no descendants in a mass screening survey of Okayama district which was performed for the past one year(1988, 4 ~ 1989,4). Hb Fukutomi, Hb A₂, Hb A₂Fukutomi and Hb F amounted in order 24.4%, 2.2%, 0.7% and 0.8% in total Hb of blood. The location of amino acid substitution in Hb Fukutomi lie in the α ₁ β ₁ contact giving rational conjecture of high oxygen affinity. Like other variants of the high oxygen affinity type this one showed increased resistance to auto-oxidation. An α tryptic peptide (α Tp-13), which is usually joined to the trypsin-resistant core of the α chain, was cleaved off by trypsin in the new variant and released into the soluble fraction as a noteworthy character of the α Fukutomi digest. The incidence of Hb variants in this area was 0.03% connoting the same frequency with other area of Japan.

B 49

THE PRIMARY STRUCTURE OF MOUSE SPHINGOLIPID ACTIVATOR PROTEIN ·

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Sphingolipid activator proteins (SAPs) are heat-stable small (8-13kDa) glycoproteins which activate several lysosomal sphingolipid hydrolases. SAPs are derived by proteolytic processing from a common large precursor. We have isolated mouse SAP precursor cDNA from liver library to investigate sphingomyelin metabolism of normal and Niemann-Pick mouse which is an excellent model for human disease. The longest clone designated as MSAP-25 was 2547 nucleotides in length with poly(A) tail at 3'-end. There was one long open reading frame which encoded 553 amino acids and the computer analysis revealed that this protein was highly homologous to rat sertoli cell glycoprotein. Similarity between these two proteins were approximately 90% which indicated these two proteins were identical. Mouse SAP also had four functional domains and each domains had nearly identical placement of cysteine residues, potential glycosylation sites, and proline residues. The heterogenities were found at the coding region and 3'-end which indicated the presence of alternating splicing in SAP mRNA.

B 50

PURIFICATION OF XERODERMA PIGMENTOSUM GROUP A COMPLEMENTING FACTOR.

Tatsuo SUGANO, Satoshi TATEISHI and Masaru YAMAIZUMI, Dept. Med. Genet., Kumamoto Univ., Kumamoto

Xeroderma Pigmentosum (XP) is autosomally inherited disease characterized by hypersensitivity to ultraviolet light and high incidence of skin cancers. XP cells are defective in the repair of ultraviolet light-induced DNA damages. So far 8 genetic complementation group (Group A-H) have been reported in XP. We have detected a group A XP complementing factor (XP-ACF) in calf thymus extract and partially purified through several colum chromatographic steps and determined some characters of XP-ACF. The molecular weight of XP-ACF was 70-90 KDa when determined by non-denatured gel filtration, 25 KDa in glycerol gradient, and 43 KDa when recovered from SDS-PAGE gels. XP-ACF was also recovered from isoelectric focusing gels in the presence of 8.5M urea. Then XP-ACF recovered from SDS-PAGE was examined on two dimentional electrophoresis.

B 51

RESIDUAL CATALASE IN THE BLOOD OF JAPANESE ACATALASEMIA·Masana OGATA·(Dept. of Public Health Okayama Univ, Sch of Med, 2-5-1 Shikata-cho, Okayama City)

The heat stability, isoelectric point and subunit size of residual catalase molecules in Japanese acatalasemia are similar to those in normal catalase, which corresponds to the fact that acatalasemia is caused by splicing mutation (Wen et al, 1990) and residual catalase is leaky type of mutation. Comparison of residual catalase in Japanese and Swiss acatalasemias suggests that both acatalasemias are derived from different origins.

In contrast to this, the properties of residual catalase in the acatalasemic mice exhibit increased PI value and lower specific activity compared with catalase in normal mice.

B 52

ESTABLISHMENT OF 3T3 CELL LINES FROM EMBRYOS OF A MOUSE MODEL FOR NIEMANN-PICK DISEASE. Kousaku OHNO, Taeko YORITA, Kenzo TAKESHITA¹⁾ and Shigeki MIYAWAKI²⁾, ¹⁾Division of Child Neurology, Tottori University School of Medicine, Yonago 683 and ²⁾Research Laboratories, Nippon Shinyaku Co., Ltd. Kyoto 601.

A strain of mice bearing an autosomal recessive gene, *spm*, has been described by Miyawaki et. al.(1982) and T.Sakiyama et. al. (1982). On the basis of clinical and pathological findings these mice have been suggested as a useful model of human Niemann-Pick disease. Recently, biochemical similarities to Niemann-Pick disease type C have been suggested (T.Sakiyama et. al., 1988, T.Tokoro et. al., 1989). We have established cell lines from homozygous (*spm/spm*) and heterozygous (*spm/+*) embryos according to a 3T3 transfer method by Todaro and Green (1963). Cloned cells from a *spm/spm* 3T3 cell line showed a extensive accumulation of intracellular cholesterol by a filipin staining as the primary cell cultures did, and were easily differentiated from cloned *spm/+* 3T3 cells. Chromosomal analysis of these cloned cells showed near tetraploidies. These immortalized 3T3 cell lines should be useful for genetic analysis of human and mouse lysosomal cholesterol storage disorder(s) and for cloning of a human gene homologous to the mouse *spm* gene.

B 53

ANALYSIS OF THE SPARSE-HAIR PHENOTYPE USING CHIMERIC MICE.

Yukiko SAKAI¹, Kazuko HAYASHI¹, Masaaki MOROHASHI², Zen-ichi OGITA¹.
(¹Dept. Patho-Biochem., ²Dept. Darm., Toyama Med. Pharm. Univ., Toyama)

Sparse-hair mice, *somousho* arose spontaneously in a stock of sib-mated albino ddY mice in our laboratory. The coat of *somousho* starts losing at 3 weeks after birth and becomes progressively thinner with age. The lost hairs are not regrowth. Viability and fertility of *somousho* are normal.

On the basis of studies of genetic cross between *somousho* and normal ddY mice, we have identified *somousho* phenotype as autosomal recessive inheritance.

As the first step to clarify the molecular lesion of the sparse-hair phenotype, we constructed chimeric mice (C57BL/6 ↔ *somousho*) by aggregation method.

We have obtained several chimeric mice. The white hairs from *somousho* background were not lost when the major population of hair-follicle cells surrounding the *somousho* hairs were derived from C57BL/6 mice.

These results suggest that introduction of hair-follicle cells from C57BL/6 into mature or fetus mice of *somousho* could restore the loss of hairs in *somousho* mice.

B 54

DETECTION OF *IN VIVO* SOMATIC MUTATIONS AT THE APRT LOCUS IN HUMANS BY THE CLONING OF PERIPHERAL BLOOD T CELLS: Masayuki HAKODA, Sanae OTSUKA, Naoyuki KAMATANI (Institute of Rheumatology, Tokyo Women's Medical College)

X-linked HPRT locus has been most commonly used to investigate the mechanisms of somatic mutations occurring *in vivo* in humans, and to determine the frequency of the somatic mutant cells. However, chromosomal events such as mitotic recombination and nondysjunction, which are thought to be important in human carcinogenesis, cannot be detected on the HPRT locus. To overcome this drawback, we have established a new method to detect mutational events at an autosomal locus, APRT. Thus, APRT-deficient mutant T cells were selectively cloned using an adenine analogue, 2,6-diaminopurine (DAP) from the peripheral blood of heterozygous individuals for APRT deficiency. It was revealed that the frequency of the mutant cells was 1.3×10^{-4} , the value one order magnitude higher than that at the HPRT locus. Complete deletion of normal alleles was observed in approximately 80% of mutant clones. Since deletion has been observed in less than 10% of HPRT mutants, it was indicated that the mutational events at an autosomal locus APRT occur in a different way from those at the X-linked locus HPRT. We could also detect two APRT mutant clones among a total number of 3.7×10^9 mononuclear cells obtained from 432 normal individuals.

C 1

STUDY ON THE ENVIRONMENTAL FACTORS HAVING AN EFFECT
ON THE STRUCTURE OF DISEASES - FROM THE STUDY ON
OKINAWAN-AMERICAN AND OKINAWAN.

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It is widely recognized that the development of specific diseases - hypertension, ischemic heart disease and diabetes mellitus are influenced by the environmental factors. To evaluate the interaction between heredity and the environmental factors, we compared the incidence of above mentioned specific diseases in Okinawan-American living in Hawaii with Okinawan.

The incidence of hyperlipidemia and ischemic heart disease in Okinawan-American was significantly higher compared with those in Okinawan. On the other hand, the incidence of hypertension was significantly low in Okinawan-American. These causes may be principally due to the difference of food composition.

C 3

POPULATION STUDIES ON THE DALMATIAN ISLANDS: ISONYMY AND
MIGRATION

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The population structure of the Dalmatian islands (Yugoslavia) is estimated using surname, marital migration and sociocultural data.

The results show that the village populations of these islands have remained essentially isolated from each other and from the mainland for several centuries. Historical and sociocultural data indicate that systematic population pressure mostly came from the coastal area of the Balkan peninsula.

Temporal trends in inbreeding and the observed differences in mating structure between subpopulations are due to the size of the local breeding population with deviations being explained in terms of emigration and/or immigration, and partly by historic and socio-economic reasons.

C 8

GENETIC POLYMORPHISM OF FACTOR I IN THE JAPANESE POPULATION: ALLELE FREQUENCIES IN IWATE PREFECTURE, NORTHERN JAPAN.

Shigeki NAKAMURA¹, Keiichi OMOTO², Akiko SAWAGUCHI¹ (¹Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo, ²Dept. Anthropol., Univ. Tokyo, Tokyo)

Polyacrylamide gel isoelectric focusing (PAGIEF) of neuraminidase treated serum samples at pH7-9 with 1.0M urea followed by semi-dry horizontal electroblotting with enzyme immunoassay was done for the detection of factor I (IF) allotypes in 270 unrelated healthy blood donors living in Iwate prefecture, northern Japan. In these samples three common types and one rare variant were observed. Three common types were A, AB and B, and a rare variant which had major band in more acidic region than A was IF A2. The allele frequencies were estimated as IF*B=0.8963, IF*A=0.1019, and IF*A2=0.0018, respectively. The data of IF allele frequencies thus far reported in Japan excluding Okinawa Islands were compared. Although the number of population samples were small, the statistically significant ($P<0.01$) geographical cline was detected for IF*A and IF*B alleles. So, the IF system is a useful genetic marker for human genetics and anthropological studies.

C 9

TRANSFERRIN POLYMORPHISM IN JAPANESE POPULATIONS: NORTH-SOUTH CLINE IN THE DISTRIBUTION OF THE C2 ALLELE. Masao NAKANAGA¹, Toshihiro YASUDA², Daita NADANO², Norio FUJIKI¹ and Koichiro KISHI² (¹Dept. Int. Med. & Med. Genet., and ²Dept. Legal Med., Fukui Medical School, Matsuoka-cho, Fukui)

Allele frequencies for human transferrin (TF) subtypes were determined using serum samples from Japanese living in Fukui prefecture, Japan, and compared with other Japanese populations using isoelectric focusing (IEF) and immunoblotting. The application of IEF revealed considerable heterogeneity in the TF system, enhancing its potential value for anthropological and genetic studies. So far, TF subtypes of about 26,000 Japanese individuals from 35 population groups have been analyzed to evaluate the degree of genetic variation at the TF locus. Possible geographical and biological factors are discussed.

C 10

HUMAN PEPSINOGEN C POLYMORPHISM. Takeshi AZUMA, Yasuki HABU, Keiichi KAWAI and R. Thomas TAGGART. Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan, Department of Molecular Biology and Genetics, Wayne State University, Detroit, Michigan, U.S.A.

Pepsinogen C (PGC) is the inactive zymogen precursor of gastricsin. Recently, we have been reported the cloning of human PGC gene. The PGC gene has been localized to 6p21.1 -> pter by analysis of somatic cell hybrids. The present study was carried out to examine the polymorphism of PGC gene.

A restriction fragment length polymorphism (RFLP) was detected with EcoRI, BamHI, PstI, SacI, and MstII by using PGC cDNA clone (PGC401). A 100-bp difference in size between the allelic fragments was found with all of these enzymes. The large or small fragments (alleles) revealed by digestion with these enzymes were coordinately present in 40 unrelated individuals. It seemed likely that the rearrangement represented an insertion or deletion of 100 bp. The region containing the polymorphism was localized within a 312 bp MspI fragment located within the intron between exon 7 and exon 8 by restriction map analysis. Sequence analysis revealed a (AT)n(GA)n repetitive region that flanked the polymorphic region. The allele frequencies of the large and small fragments estimated from 40 unrelated Japanese were 0.806 and 0.194, respectively.

C 11

HUMAN SERUM DEOXYRIBONUCLEASE I POLYMORPHISM: PATTERN SIMILARITIES AMONG ISOZYMES FROM SERUM, URINE, KIDNEY, LIVER, AND PANCREAS. Daita NADANO, Toshihiro YASUDA and Koichiro KISHI (Department of Legal Medicine, Fukui Medical School, Matsuoka-cho, Fukui)

A combination technique of polyacrylamide-gel isoelectric-focusing electrophoresis and a newly developed zymogram method have led to the discovery of genetic polymorphism of human serum deoxyribonuclease I (DNase I). Family studies showed that the three common phenotypes—DNASE1 1, DNASE1 1-2, and DNASE1 2—and the other five relatively rare phenotypes—DNASE1 1-3, DNASE1 2-3, DNASE1 2-4, and DNASE1 3-4—represent homozygosity or heterozygosity for four autosomal codominant alleles, DNASE1*1, DNASE1*2, DNASE1*3, and DNASE1*4. The frequencies of DNASE1*1, DNASE1*2, DNASE1*3, and DNASE1*4 calculated in a Japanese population were 0.5517, 0.4358, 0.0104, and 0.0021, respectively. Moreover, it was found that urine and extracts of kidney, liver, and pancreas, as well as serum, can be used for DNase I phenotyping.

Kishi, K. et al. (1990) Am. J. Hum. Genet., 47, 121-126

C 12

Detection of a Mongoloid-specific 9-bp deletion of mitochondrial DNA. Satoshi HORAI and Kumiko MURAYAMA
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Mishima.

Using the RFLP of mitochondrial DNA, we first showed that the Japanese population could be separated into two distinct groups : A group with smaller frequency (group I) first diverged from the other group with larger frequency (group II). The majority of the group I show HaeIII-morph 2, which is characterized by deletion of one copy of a 9-bp tandem repeat sequence in a small noncoding region (region V). This deletion is found only in Mongoloids. With employing the polymerase chain reaction, DNAs from Taiwan Chinese and Taiwan aborigine were analyzed. We observed an amplified fragment of 100-bp or 91-bp derived from region V, which corresponds to the presence of two or one copy, respectively, of the 9-bp sequence, in each individual. Overall, 17% of Taiwan Chinese and 28% of Taiwan aborigine have the 9-bp deletion in region V. Published information on the frequency of the deletion in several Asian and Pacific populations is compared with the present data to elucidate a feature about distribution of the deletion in Mongoloid populations.

C 13

NINE BASE PAIR DELETION OF MITOCHONDRIAL DNA AMONG ASIAN POPULATIONS. Shinji HARIHARA, Koji SHIMIZU (Inst.Dev.Res., Aichi Pref.Colony, Kasugai-shi, Aichi), Yumiko SUUTO, Momoki HIRAI, Keiichi OMOTO (Dept.Anthrop., Fac.Sci., Univ.Tokyo, Bunkyo-ku, Tokyo)

Individuals of five Asian populations (51 Ainu, 63 Japanese, 64 Koreans, 37 Negritos and 20 Vedda), were surveyed for the presence of 9-bp deletion of mitochondrial DNA (mtDNA). The variation, which was reported to be east-Asian specific and was detected with extremely high frequencies in Polynesian populations, could be distinguished from the normal type by PCR and polyacrylamide gel electrophoresis. The frequencies in the populations were: 2.0% in Ainu, 19.0% in Japanese, 7.8% in Korean, 91.9% in Negrito and 0.0% in Vedda. Among each two populations, the distribution of the deletion type was compared statistically by χ^2 -test. Negrito and the any of the remaining populations, and Japanese and Ainu are different in the distribution. Combining our previous results on mtDNA RFLPs, a total number of mtDNA types found in the five Asian population became 22. The deletion type of mtDNA can be called a marker for Southern Mongoloid populations and also may be useful in investigating migration of Asian populations in the past.

C 15

COAGULATION FACTOR XIII B, PLASMINOGEN AND COMPLEMENT ALLOTYPES IN JAPANESE PATIENTS WITH PRIMARY VARICOSE VEINS. Kenji MIZUTANI¹, Hiroaki NISHIMUKAI², Kanji IWAHASHI¹, Kengo TSUNEKAWA¹, Takaaki SHINOMIYA² (¹Dept. 1st Surg., ²Dept. Legal Med., Sch. Med., Ehime Univ., Ehime)

Primary varicose veins (PVV) are permanent dilatation and tortuosity of veins, and are most often seen beneath the skin of the legs. Though PVV is known as a familial disease, the mode of inheritance is obscure. The genetic factors in the development of PVV are important. The present study reports the genetic polymorphisms of the B subunit of coagulation factor XIII (F13B), plasminogen (PLG), complement C6, C7, factor B (BF) and factor I (IF), among Japanese patients with PVV (n=21). The allotypes of these factors were studied by the method of isoelectric focusing followed by immunoblotting. The allele frequencies for F13B*2 and IF*A among PVV patients were significantly higher (F13B*2, $p=0.0047$; IF*A, $p=0.0006$) than those in healthy controls (n=60). Significant associations of F13B 2 allotype [$p=0.0220$, relative risk (RR)=13.9] and IF A allotype ($p=0.0006$, RR=10.0) with PVV were observed, however, no significant association of PLG, C6, C7 or BF allotype with the disease was found.

C 16

COAGULATION FACTOR XIII B AND PLASMINOGEN ALLOTYPES IN JAPANESE PATIENTS WITH BULLOUS PEMPHIGOID AND PEMPHIGUS. Hiroaki NISHIMUKAI¹, Takaaki SHINOMIYA¹, Koji SAYAMA², Yuji SHIRAKATA², Satoshi SHIRAIISHI², Yoshiharu MIKI² (¹Dept. Legal Med., ²Dept. Dermatol., Sch. Med., Ehime Univ., Ehime)

The allotypes of the B subunit of coagulation factor XIII (F13B) and plasminogen (PLG) were studied in Japanese patients with bullous pemphigoid (BP; n=17) and pemphigus [P; n=11 (5 with pemphigus vulgaris and 6 with pemphigus foliaceus)], and in 60 healthy adults to investigate the genetic background of these diseases. The allotypes were examined by using agarose gel isoelectric focusing followed by immunoblotting. The frequency for F13B 3-1 phenotype among P patients (63.6%) was significantly higher ($p=0.019$) than that in BP patients (17.6%), and the frequency for F13B 3 phenotype among BP patients (70.6%) was significantly higher ($p=0.009$) than that in P patients (18.2%). The allele frequencies for F13B*1 in BP and P were 0.2059 and 0.5000, respectively ($p=0.023$). The present study suggests that BP is associated with F13B-3 allotype (or F13B 3 phenotype) and P is associated with F13B-1 allotype (or F13B 3-1 phenotype). No significant association of PLG allotype with P or BP was found.

C 17

ANALYSIS OF T CELLS INVOLVED IN AN IMMUNE SUPPRESSION MEDIATED BY HLA-DQ MOLECULE

Hideyuki YOSHIZUMI¹⁾, Nobuhiro KAMIKAWAJI¹⁾, Ko OKUMURA²⁾, Yasuharu NISHIMURA¹⁾, and Takehiko SASAZUKI¹⁾ (1)Dept. of Genetics, Med. Inst. of Bioreg., Kyushu Univ., Fukuoka, (2)Dept. of Immunol., Juntendo Univ., Tokyo, Japan)

Low responsiveness to streptococcal cell wall antigen(SCW) is controlled by an HLA-linked immune suppression gene. We previously reported the existence of HLA-DQ restricted CD4⁺ T cells in low responders to SCW, which promoted proliferation of CD8⁺ $\alpha\beta$ TCR⁺ ($\alpha\beta$ CD8) T cells with immunosuppressive activity, we established $\alpha\beta$ CD8 T and CD4⁺ $\alpha\beta$ TCR⁺($\alpha\beta$ CD4) T cell lines respectively from a low responder to identify the specificities of these T cells. It was revealed that the most of DQ-restricted $\alpha\beta$ CD4 T cells expressed TCR V β 5.3 and that $\alpha\beta$ CD8 T cells expressed V α 2 and V β 5.2 by the analysis with anti-TCR monoclonal antibodies. The DQ restricted $\alpha\beta$ CD4 T cells recognized streptococcal M protein in the context of HLA-DQ. The $\alpha\beta$ CD8 T cells required IL2 and autologous monocytes but not SCW to proliferate. Moreover, their growth was accelerated when monocytes were pretreated with IFN- γ . The $\alpha\beta$ CD8 T cells contained perforin and lysed K562 cells in the presence of anti-CD3 monoclonal antibody, but did not lyse $\alpha\beta$ CD4 T cells. Our results indicate that immunosuppressive $\alpha\beta$ CD8 T cells generated from a low responder proliferates by recognizing unknown molecules expressed on autologous monocytes probably through TCR V α 2+V β 5.2 and that $\alpha\beta$ CD8 T cells have a potential to lyse target cells.

C 18

HLA-DQ restricted immune recognition of streptococcal antigen in low responders.

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We have found an HLA-linked immune suppression gene to streptococcal cell wall antigen(SCW) through the induction of CD8⁺ suppressor T cells. 80 healthy donors were divided into high and low responders to SCW and HLA-DQ alleles of them were determined by DNA typing. HLA-DQA1*0102, DQA1*0103 or DQB1*0601 was increased in frequency in the low responders and the frequency of DQA1*0301, DQB1*0401 or DQB1*0303 was increased in the high responders. The heterozygotes of DQA1*0102 or 0103 and DQA1*0301 showed a low response to SCW, thereby confirming that the HLA-linked gene controls the low response, as a dominant genetic trait. Using mouse L cell transfectants as an antigen presenting cell, we found that DQw6(DQA1*0103, DQB1*0601) activated SCW specific T cells, whereas DQw4(DQA1*0301, DQB1*0401) did not. DR4 and DR2 presented SCW to CD4⁺ T cells in both the high and low responders to SCW. Thus in low responders to SCW, DQ restricted CD4⁺ T cells specific to SCW present and might down regulated the immune response to SCW restricted by DR through the induction of CD8⁺ T cells.

C 19

SEQUENCE VARIATION IN HLA-DRw8 SPECIFICITY. Yoshihisa WATANABE¹, Takao KOHSAKA¹, Noboru KOBAYASHI², Katsushi TOKUNAGA³, Takeo JUJI³ (¹Dept. Immunol., Nat. Child. Med. Res. Cent., Tokyo, ²Nat. Child. Hosp., Tokyo, ³Blood Transfusion Serv., Univ. of Tokyo, Tokyo)

The nucleotide sequence of a DRB1 gene of DRw8-DQw1(6), which is a predominant subtype of DRw8 in Japanese, was determined with single stranded DNA enzymatically amplified by polymerase chain reaction (PCR) and compared with those of published DRw8 subspecificities.

The sequence differs at a single amino acid from both of the published DRw8/Dw8.1 and DRw8/Dw8.2 sequences: Ile⁶⁷(ATC) instead of Phe⁶⁷(TTC) in DRw8/Dw8.1 and Ser⁵⁷(AGC) instead of Asp⁵⁷(GAT) in DRw8/Dw8.2. On the other hand, it has the same amino acid sequence as that of DRw8/Dw8.3 although one nucleotide difference has been observed between the two sequences. These results indicate that Japanese DRw8-DQw1(6) corresponds to DRw8/Dw8.3. Furthermore, this was confirmed by the PCR-SSO method with an oligonucleotide probe specific for the DRw8-DQw1(6) sequence. This probe clearly distinguished the subspecificities.

The nucleotide sequences of the other subspecificities, DRw8-DQw3(8) and DRw8-DQw4, were partially determined by the cloning method after amplification. At the 57th and 67th amino acid position, they have the same amino acids as those of DRw8/Dw8.2.

C 20

MUCOPOLYSACCHARIDOSIS TYPEW; CHARACTERIZATION OF MUTATION AND ITS MOLECULAR HETEROGENEITY. Shunji TOMATSU, Seiji FUKUDA, Kazuko SUKEGAWA, Toshiya SASAKI, Hiroyuki OKAMOTO, Tadao ORII, (Dept. of Pediatrics, Gifu University, School of Medicine, Gifu)

We have identified several mutations causing β -glucuronidase (β -GI) deficiency in three cases with mucopolysaccharidosis typeW (MPSW). Enzyme assay of lysates of these lymphocytes or cultured fibroblasts showed little residual activity and that the β -GI specific mRNA levels were normal, as revealed by Northern blot analysis. Mutated cDNA clones including the entire coding sequencing were isolated from a library in case 1 and PCR (polymerase chain reaction) in case 2 and 3 derived from cultured fibroblasts. Sequencing of the full-length mutated cDNA revealed C→T transitions, an event causing a single Ala⁶¹⁹→Val change (cases 1 and 2) and Arg³⁸²→Cys and Pro⁶⁴⁹→Leu changes (case 3). The former change is detected by loss of the cleavage site for the enzyme FNU 4 HI restriction site, the patients (case 1 and 2) were shown to be a homozygote with this mutation and the parents and brother in case 1 were heterozygotes. The Ala⁶¹⁹→Val and Arg³⁸²→Cys mutations disrupt a functional domain consisting of a region of sequence highly conserved among human, rat and bacterial β -GI's, and they reduce the enzyme activity, as tested by transfection of COS cells with expression vectors harboring the mutated cDNA. But the Pro⁶⁴⁹→Leu mutation does not reduce the enzyme activity.

C 22

CONSTRUCTION OF A GIANT FRAGMENT-SPECIFIC CLONE LIBRARY USING FLOW-SORTED HUMAN CHROMOSOMES AND SHOTGUN PCR. Shinsei MINOSHIMA, Kazuhiko KAWASAKI, Jun KUDOH, Ryuichi FUKUYAMA, Kosuke SAKAI and Nobuyoshi SHIMIZU (Department of Molecular Biology, Keio University School of Medicine)

We have developed a method to construct a clone library specific to a particular region of a human chromosome using shotgun PCR and flow-sorted chromosomes. These clones may facilitate physical mapping of the selected region and isolation of unique sequences or potential disease-related genes within that region. We have applied this shotgun PCR method to a large region of chromosome 22 including the Ig λ gene. Metaphase chromosomes were prepared by the polyamine-digtonin method from the human B-lymphoblastoid line GM130B stained with propidium iodide and sorted by a FACS440 sorter. Chromosome 22 was collected in an intact form, embedded in a low temperature-melting agarose block and treated successively with proteinase K and the restriction enzyme NotI. DNA fragments were separated by pulsed field gel electrophoresis. A region corresponding to giant NotI DNA fragments (1.0 Mb-2.5 Mb) was sliced into nine sections and a fraction containing the Ig λ (Ke⁻/Oz⁺) marker was identified by the PCR method. This fraction was subjected to Alu-PCR or ligation-mediated PCR. The amplified DNA sequences were successfully cloned into plasmid vectors. These clones are being tested for use in screening genomic libraries and physical mapping of the Ig λ -containing region.

C 23

CONSTRUCTION OF NO.18 CHROMOSOME SPECIFIC GENE LIBRARY. Hitoshi NAKASHIMA, Asao FUJIYAMA, Tomoko HASEGAWA*, Takashi IMAMURA (Dept. Hum. Genet., Natl Inst. Genet, Mishima; *Shizuoka Children Hospital, Shizuoka)

Construction of a human genetic linkage map will greatly facilitate the identification and characterization of chromosome loci involved in inherited disorders. As part of our genome-mapping effort, we undertook a systematic approach for construction and use of a series of pWE15 cosmid and a Charon 21a EcoRI libraries specific for No.18 chromosomes. High molecular weight genomic DNA for cosmid cloning was prepared from a human-mouse hybrid cell line (kindly donated by Dr. M. Hori) containing No.18 and a part of X-chromosomes as the human chromosome. Approximately 2.0×10^5 independent colonies were screened using 32-P-labeled total human DNA as a probe, yielding 1.2×10^4 clones. Also, recombinant phages from a ATCC No. 57742 library containing inserts devoid of highly repetitive sequences were isolated using labeled total human DNA as a probe. The phage particles were purified through DE52 chromatography, and the DNA isolated and subcloned into pUC19 plasmid vector. The resulting colonies that failed to hybridize were picked and purified through at least two rounds of plating and rehybridization. Approximately 2,500 colonies yielded 1,595 subclones containing unique inserts. Non-radioactive in situ hybridization was carried out using these inserts DNA as probes, which tested for their ability to detect sequences specific for the chromosome 18.

C 24

EXPRESSION OF HUMAN LACTATE DEHYDROGENASE-C GENE IN TRANS-GENIC MICE. Keiko MOMOI, Clement L. MARKERT (Dept. Animal Science, North Carolina State Univ. Raleigh, NC) and Ken-ichi YAMAMURA (Inst. Med. Genet. Kumamoto Univ. Med. School, Kumamoto)

To investigate the genetic and physiological significance of isozymes, we have generated two kinds of transgenic mouse lines, harboring the human LDH-C coding region attached to the mouse metallothionein-I (MT-I) promoter or to the cytomegalovirus enhancer and the chicken cytoplasmic β -actin promoter. The native LDH-C gene is expressed in males only in primary spermatocytes. It is not expressed at all in females. The metallothionein gene is expressed in most tissues. The cytoplasmic β -actin gene is expressed in all non-muscle cells. We therefore expected the human LDH-C constructs to be expressed in most transgenic tissues. The human LDH-C gene coupled to the β -actin promoter was expressed in varying amounts in a wide range of tissues of both male and female transgenic mice, especially muscle and heart. LDH zymogram analysis suggested that human LDH-C was expressed more than endogenous mouse A gene in transgenic muscle. Surprisingly, the human LDH-C gene coupled to the MT-I promoter was expressed only in the testes in all transgenic lines. Further analysis will be required to clarify the mechanisms controlling gene expression in these transgenic mice and to assess the physiological effect of additional LDH-C isozymes.

C 26

INHERITED QUANTITATIVE DNA VARIATION IN THE "APOLIPOPROTEIN(A)" GENE. I. KONDO AND K. BERG (INSTIT. OF MED. GENET. UNIV. OF OSLO, OSLO, NORWAY).

The Lp(a) antigen resides in a polypeptide chain that is attached to apolipoprotein B(apoB) by a disulfide bridge. Recently, cDNA for this polypeptide chain (frequently referred to as the Lp(a) polypeptide chain, Lp(a) apolipoprotein or apolipoprotein(a) was cloned. We have studied a variant 2kb DNA fragment detected after digestion with the restriction enzyme, MspI, and it is related to the "kringle IV" region of the Lp(a) gene, since it is detected with Lp(a) probe that only contains "kringle IV" repeats. A proportion of people appears to lack (or have an undetectable level of) the 2 kb fragment and there are significant quantitative differences between samples from people who have the fragment. Presence and amount of this fragment appear to segregate in families as a Mendelian trait. This quantitative DNA segregation in families as a Mendelian trait. This quantitative DNA variation is likely to reflect differences between individuals in number of "kringle IV" repeats at the Lp(a) locus.

C 27

MOLECULAR ANALYSIS OF FAMILIAL ANIRIDIA (AN2) WITH 11p13 DELETION. Y. FUKUSHIMA¹, K. WAKUI², T. NISHIDA², Y. UEOKA³, S. IKEMOTO⁴, S. SHIMIZU⁵ (Div. Med. Genet.¹, Dept. Clinical Lab.², Div. Ophthalmol.³, Saitama Children's Medical Center, Jichi Medical College⁴, Mitsubishi BCL⁵)

The propositus (4m), the father (26y) and the paternal grandmother (64y) were affected with aniridia, cataract and glaucoma. They had normal growth and mentality, and no other anomalies. High-resolution chromosome analysis revealed a tiny interstitial deletion within 11p13 band in these three family members [46,XY or XX,del(11)(p13p13)]. Catalase activity of the patients were within normal limits. Southern blots from digests of DNA of the family members were hybridized with DNA probes 1027 and 530, which have been assigned to 11p13, and rehybridized with probe 333, mapped to 11q, for calibration. Comparing the hybridization signals of the DNA fragments of the patients and normal controls, the patients had one copy of probe 1027 and two copies of probe 530. Probe 1027 seemed to be closely linked to aniridia (AN2) gene. This is the first report of dominantly inherited aniridia affected with 11p13 deletion. This family is very useful for physical mapping of genes located near 11p13.

C 28

DETECTION OF DNA DELETION FOR EARLY DIAGNOSIS OF VON RECKLINGHAUSEN NEUROFIBROMATOSIS. Tsutomu KAMEI, Kei TAKAHASHI, Sumio MASUMURA (Shimane Med. Univ., Izumo) Yoshimitsu FUKUSHIMA, Akihiko SHIBATA, Yasuhide HAYASHI (Saitama Child. Med. Center, Iwatsuki), Naoki TACHIBANA (Aomori Pref. Cent. Hosp., Aomori), Francis S. COLLINS (Univ. of Michigan, Ann Arbor USA), Norio NIIKAWA (Nagasaki Univ., Nagasaki)

Von Recklinghausen Neurofibromatosis (NF1) is known as a common autosomal dominant disorder with high spontaneous mutation rate, but the molecular characteristics of the genetic defect has been unknown. A gene dose study related to the gene for NF1 was carried out with Southern blotting. A subcloned DNA, 17L1A, that was closely mapped to the translocation breakpoint in 17q11.2 of a NF1 patient was used as a probe. Among five NF1 patients, a density reduction by half was found in three patients, suggesting a DNA deletion involving 17L1A allele. No reductions were detected in two NF1, two Noonan-Neurofibromatosis (NFNS) patients. Therefore, it is mostly possible that the deletion related to the NF1 gene might cause this disorder, whereas NFNS would not be associated with a DNA deletion of NF1 gene. This probe will be useful for the early diagnosis of NF1, particularly in sporadic cases. Prenatal diagnosis in familial cases will be also possible with this analysis.

C 29

Variation in the organization of the rRNA gene clusters among flow-sorted human acrocentric chromosomes. Ryuichi FUKUYAMA, Shinsei MINOSHIMA, Jun KUDOH, Kazuhiko KAWASAKI and Nobuyoshi SHIMIZU. (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo)

In the human genome there are about 800 copies of rRNA genes which are organized as tandem repeat units and distributed in clusters over the short arms of all five acrocentric chromosome pairs. The structure-function relationship of these rRNA gene clusters is not yet completely understood. Therefore, we have studied this problem by the cytological and molecular techniques using flow-sorted human acrocentric chromosomes. Silver staining of the fixed metaphase chromosomes of B-lymphoblastoid line GM130B revealed accumulation of more silver grains in the D group chromosomes than the G group chromosomes, suggesting that the rRNA genes in the D group chromosomes are more actively transcribed. Fluorescence in situ hybridization (FISH) using 28S rRNA gene as a probe demonstrated that the number of rRNA genes in the G group chromosomes is larger than the D group chromosomes. These findings indicate that the copy number of rRNA gene is not directly correlated to the extent of the active transcription. Southern blot analysis using methylation sensitive restriction enzymes revealed that the rRNA genes in the G group chromosomes are methylated more than those in the D group chromosomes. This suggests that the methylation status in the rRNA genes varies among acrocentric chromosomes and it might control the transcription of rRNA genes.

C 30

BIOETHICAL CONSIDERATION IN GENETIC COUNSELLING.

Shigeaki NAKAZAKI, Mikio HIRAYAMA, Norio FUJIKI, Kazuo MANO+ and Yasuko SHIRAI++

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Since 1961, we have performed approximately 3000 cases of genetic counselling under the guidances of Late Profs. Komai and Masuda.

I would like to briefly summarize our cases, classifying by client sex, age, motive, laboratory examination, genetic problems etc, and discuss the principle of genetic counselling; informed consent, self decision making, as well as confidentiality, as recommended in our edited book of Guidebook of Genetic Counselling. We have also made followup study and analysed for counselling technique and evaluation.

After consensus survey on the handicapped and heredity for general public, we should discuss the the moral norm and make our decision on the common understanding on both newly developed and old traditional evaluation and comment more curriculum on human genetics and bioethics in medical and postgraduate stages, in order to educate general public, as emphasized at 1987 Bioethics Seminar in Fukui.

C 32

THE HUMAN GENE MAP DATABASE FOR A PERSONAL COMPUTER.

Shinsei MINOSHIMA, Jun KUDOH, Harumi HARIGAI, Minoru KANEHISA^{*} and Nobuyoshi SHIMIZU (Dept. Molec. Biol., Keio Univ. Sch. Med., ^{*}Inst. Chem. Res., Kyoto Univ.)

Over 5,000 genetic markers, including 1,631 genes, 113 fragile sites and 3,300 anonymous DNA segments, were compiled at the HGM10 workshop last year. These human gene mapping database at Yale (HGML) can be accessible from Japan through an international telephone line. However, the telephone charge is considerable and the prompt response is not always available from the HGML. Therefore, a stand-alone application system for the human gene mapping database is of great necessity.

We have attempted to establish a human gene map database, JHGM, where Japanese language can be used. The data were compiled from HGM9, HGM10 and independent searches. We used a PC-9800 series personal computer (NEC) as a hardware and Let's Iris 2.0 (Personal Media Co. Ltd.) as a software. In the current version of JHGM, the database can be searched with various items such as chromosome number, gene symbol, genetic trait, mapped region, mapping method and literature. The JHGM database will be made available to the public use.

C 33

GENE AMPLIFICATION OF N-MYC IN A NEUROBLASTOMA CELL LINE, KPNRT-BMV, H.NAKAGAWA¹, J.INAZAWA², T.TAKASHIMA¹, Y.SHIZUMI¹, S.TANAKA¹, H.NISHIGAKI¹, M.TANIWAKI¹, S.MISAWA¹, T.SUGIMOTO³, K. KASHIMA¹, T. ABE² (¹3rd Dept. Internal Med., ²Dept. Hygiene, ³Dept. Pediatrics, Kyoto Pref. Univ. Med., Kyoto)

A neuroblastoma cell line KPNRT-BMV, which was established from bone marrow of a neuroblastoma patient is known to have 3 HSRs, A 120-150 fold amplification of N-myc gene at these HSR was reported with southern blot hybridization and radioisotopic in situ hybridization. (Int J cancer, 1989) Fluorescence in situ hybridization (FISH) was performed using a biotinylated N-myc (Oncor.). Amplification of the N-myc signal was done according to the method by Pinkel et al. after chromosomal hybridization. N-myc gene amplification at HSR and interphase nuclei was detected by FITC conjugated avidin. Hybridization with biotinylated alpha satellite DNA was also performed to identify the chromosomes containing HSR. The results showed that the chromosomes with HSR and a marker chromosome of unknown origin were all rearranged chromosome 17's.

C 34

DETECTION OF i(17q) CHROMOSOME ABNORMALITY BY BIOTIN-LABELLED FLUORESCENCE IN SITU HYBRIDIZATION USING CHROMOSOME-17 SPECIFIC REPEATED DNA PROBE. S. MISAWA, H. NAKAGAWA, J. INAZAWA*, T. TAKASHIMA, Y. SHIZUMI, S. TANAKA, H. NISHIGAKI, M. TANIWAKI, K. KASHIMA, T. ABE* (3rd Dept. Int. Med. and *Dept. Hygiene, Kyoto Pref. Univ. Med, Kyoto)

Fluorescent in situ hybridization (FISH) using a biotinylated chromosome 17 specific alpha satellite DNA probe was performed to detect i(17q) in blood samples from 2 patients with chronic myelocytic leukemia in blast crisis. G-banded karyotypes of both patients showed an i(17q) as an additional chromosomal aberrations to a standard Ph. With FISH, normal chromosome 17 and i(17q) were easily detected and distinguished from other chromosome than 17 in metaphase plate and a dicentric pattern was shown. In most interphase nuclei, 2 fluorescence spots were observed. Furthermore, in some interphase nuclei, the isochromosome was also discernible by the double structure of the one of these fluorescence spots.

C 35

CYTOGENETICAL ANALYSIS OF AMNIOCYTES BY IN SITU METHOD. Ying-hua ZHANG, Fumiko SUGIURA, Shouichiro TSUDA, Tatsuo ABE (Depts of Hygiene & Med. Kyoto Pref. Univ. Med., Kyoto Inst. Microbiol.)

Thirty-four samples of amniocytes from women undergoing prenatal diagnosis of 16 to 20 weeks' gestation were cytogenetically studied in vitro. The amniocytes were prepared in Chang's medium at 2×10^5 /ml and cultured on the slide glass for one to two weeks at 37 °C and 5% CO₂ in air until colony formations were observed. The cells were harvested by adding 3-5 µg/ml colcemid 4-5 hr before fixation. The fixation was made on the same slide glass. After drying, the cells were Q- and G-banded by Qunacriner mustard and by trypsin Giemza technique, respectively. Using this newly developed in situ method, chromosome preparations of well-spread prometaphase could be obtained and banded satisfactorily in all cases. Twenty-eight cases revealed normal karyotype and six cases were abnormal. The abnormal karyotypes as follows; ①47,XY,+18 ②46,XY/47,XY,+17 ③46,XY,r(13)(p11.2→q34) ④46,XY,t(13;14)(p11;q11.1) ⑤46,XY,t(2;3)(p13;q27)/47,XY,t(2;3)(p13;q27),+mar ⑥46,XY,t(22;?)(p11;?). This in situ method provide us chromosome preparations of high quality compared with conventional methods.

C 36

BIOTIN-LABELLED CHROMOSOME MOLECULAR HYBRIDIZATION DETECTION WITH IMMUNOGOLD SILVER AMPLIFICATION. Keiichi MIYAGOE¹, Masafumi TANIWAKI², Johji INAZAWA², Hitoshi NAKAGAWA², Shinji TANAKA², Shinichi MISAWA², Kei KASHIMA², Tatsuo ABE², Toru SUGIMOTO²
(¹Kyoto Inst. Microbiol., ²Kyoto Pref. Univ. Med.)

We describe a method of chromosomal in situ hybridization using a biotinylated probe which was detected by silver amplification of immunocytochemical reaction product. Probes hybridized to chromosomes obtained from a neuroblastoma cell line (RT3) and PHA-stimulated lymphocyte; N-myc (1.0Kb EcoRI-Bam HI fragment) for RT3 cell and D1Z2 (1.9Kb Hind III fragment) for lymphocyte. HSRs in RT3 were labelled prominently with silver grains. By using D1Z2 probe silver grains were observed at centromeric regions of Nos. 1, 9, 15. Under the hybridization condition used in this study, D1Z2 showed minor hybridization sites on several other chromosomes.

C 37

Assignment of calpastatin gene (CAST) to chromosome 5 at the region q14-q22 : Johji INAZAWA, Hitoshi NAKAGAWA, Shinichi MISAWA, Tatsuo ABE (Depts. of Hygn and Med., Kyoto Pref. Univ. of Med.), Shinsei MINOSHIMA, Ryuichi FUKUYAMA, Nobuyoshi SHIMIZU (Dept. of Mol. Biol. Keio Univ. School of Med.), Masatoshi MAKI, Masakazu HATANAKA, Takashi MURACHI (Inst. for Virus Res. and Dept. of Clinical Sci. and Labo. Med., Facul. of Med., Kyoto Univ.)

Calpastatin is an endogeneous protein inhibitor acting specifically on calpain (EC 3.4.22.17; Ca²⁺-dependent cysteine protease). Both calpain and calpastatin are widely distributed in mammalian and avian cells. Recent success in cloning of both the cDNA and genomic DNA fragment for human calpastatin revealed that this gene consists of four internal repetitive domains and non-homologous N-terminal domain. The chromosomal locus of the human calpastatin gene (CAST) was assigned to chromosome 5 by spot-blot hybridization analysis with flow sorted chromosomes. Further, it was sublocalized to bands 5q14-q22 by in situ hybridization to metaphase chromosomes using a cDNA probe encoding the 5'-terminal region of CAST.

C 38

FAMILY STUDIES OF THE LDL RECEPTOR GENE OF HEREDITARY

HYPERCHOLESTEROLEMIA ASSOCIATED WITH ACHILLES TENDON XANTHOMAS

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and Hideo HAMAGUCHI¹ (¹Dept. Hum. Genet., Inst. Basic Med. Sci.,
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To assess the relationship between relatively severe hereditary hypercholesterolemia with Achilles tendon xanthomas and the defect of the LDL receptor gene, family studies were carried out in 17 hypercholesterolemic families. In 16 out of the 17 families, hypercholesterolemia was co-segregated with gross rearrangement, RFLP, or an abnormal TaqI band of the LDL receptor gene. This finding is compatible with the interpretation that hypercholesterolemia is due to defective LDL receptor genes. In the 16 pedigrees, co-segregated genetic markers of the LDL receptor gene included four different deletion mutations, six different RFLP haplotypes and one TaqI abnormal band, suggesting that the origin of the mutant LDL receptor genes in Japanese generally differs among different pedigrees. In the one other family, the proband and his sibling, both having relatively severe hypercholesterolemia and Achilles tendon xanthomas, shared an RFLP haplotype, although the proband's other sibling with moderate hypercholesterolemia but without Achilles tendon xanthomas did not. These data indicate that most, if not all, of the relatively severe hereditary hypercholesterolemia associated with Achilles tendon xanthomas is caused by a defect of the LDL receptor gene.

C 39

COMPLEMENTATION STUDY OF INHERITED PEROXISOME-DEFICIENT DISORDERS

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Genetic heterogeneity in peroxisome-deficient disorders, including Zellweger syndrome(ZS), neonatal adrenoleukodystrophy(NALD) and infantile Refsum disease(IRD) was investigated. [Methods] Fibroblasts were fused with polyethyleneglycol and the formation of peroxisomes was visualized by immunofluorescence staining using anti-catalase IgG. Peroxisomal β -oxidation was estimated by lignoceric acid oxidation. Biosynthesis of peroxisomal enzymes was analyzed by pulse-chase experiments. [Results] In the complementary matching, peroxisomes were formed and lignoceric acid oxidation was restored. Processing of peroxisomal proteins was also normalized. Five complementation groups were identified. Group A: ZS and IRD; Groups B, C and D: ZS; Group E: ZS, NALD & IRD. [Conclusion] Five genotypes are present in patients with deficient peroxisomes. Clinical phenotypes do not correlate with the genotypes. These results also indicate that at least five different genes are involved in the formation of normal peroxisomes and in the transport of peroxisomal enzymes.

C 40

A CASE OF INCOMPLETE HEREDITARY METHEMOGLOBINEMIA ASSOCIATED WITH PARTIAL CYTOCHROME b₅ REDUCTASE DEFICIENCY IN NONERYTHROID CELLS
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A case of incomplete hereditary methemoglobinemia associated with partial NADH-cytochrome b₅ reductase deficiency in nonerythroid cells was reported in a 43 year-old Japanese male, who had grey cyanosis, neurological and mental retardation, and developmental disorders as major clinical symptoms.

Methemoglobin reductase activity in erythrocytes determined as NADH-ferricytochrome b₅ reductase by Hegesh was essentially defective, while the activities in lymphocytes and platelets determined as NADH-diaphorase, NADH-ferricyanide reductase, NADH-ferricytochrome b₅ reductase and NADH-cytochrome b₅ reductase using 2,6-dichlorophenol-indophenol, potassium ferricyanide, ferrihemoglobin and cytochrome b₅ as substrates, respectively, were nearly 1/4 as compared to those of normal ones.

This case resembles clinically generalised type of hereditary methemoglobinemia (Type II) by Jaffé, but not similar in a profile of enzyme deficiency in nonerythroid cells, and neurological and mental manifestations were not lethally severe.

C 42

FURROWS AND DERMAL RIDGES OF THE HAND IN A CHILD WITH FETAL ALCOHOL SYNDROME

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We reported a 2 year-old female patient with severe fetal alcohol syndrome, whose mother, Filipina, had been drinking hard in early pregnant period. The patient had growth and mental retardation, severe hydrocephalus and typical craniofacial dysmorphism. The anomalies of dermatoglyphics in this patient were as follows; (1) Palmar creases: the interdigital part of the distal palmar crease was sharply bent and the proximal transverse crease was hypoplastic. A subtype of simian crease was observed in her left hand. (2) Patterns of the fingertips: whorls in eight fingers and loops in two were observed. This pattern was similar to her mother's.

C 43

A case with 47, XX, +der(22), t(11;22)mat: Mitsushiro KIDA(1), Eiko ARAI(2), Kumiko KOMAKI(3), (1)Dept. Ped. Teikyo Univ., (2)Dept. Cytogenetics Tokyo Dent. Med. Univ., (3)Isshin Hosp.)

A female infant 4 months old was presented for scrutiny with delayed development and multiple anomaly as main complaints. According to a letter of introduction, 41 weeks of fetus period, normal delivery, and 2600g of body weight at birth had been found, and she had a small lower jaw and weak suckling power from birth while being raised through a catheter for 3 days. At present, she growth well physically with 5850g of body weight and 62 cm of height; but she does not show a fixed cervix, laughter at being amused, or attention to object (attention to light), having larynges stridor, small lower jaw, and dislocation of hip joint. The symptoms in the patient include severe mental and motor developmental delay, reduced muscular tension, broad face, fatty cheek, deformation of auricle, burrow of anterior auricle, widely opened eyes, strabismus, short, wide and beaked nose, long philtrum, cleft palate, small jaw, retracted lower jaw, hypoplasia of hip joint, clubfoot, long fingers, depressed scutum. Examination of chromosomes revealed 47, +der(22), t(11;22)(q23;q11)mat in the patient., 46, XY in the father, and 46, XX, t(11;22)(q23;q11) in the mother.

C 44

The analysis of weight and height of twins at birth and one year of age.

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Body weight and height of 558 pairs of twins at one year of age was analyzed in comparison with that at birth. As to both weight and height, no significant difference with regard to zygosity and birth order was observed at one year of age. On the contrary apparent sex difference was seen in both weight and height of one year of age. As to the distribution of weight and height, positively changed skewness was demonstrated, if compared with that at birth. It was indicated that the lower the weight or height at birth, the higher the growth rate of weight or height. This results means that obvious recovery of weight and height was made in this period. Increased intraclass correlation coefficient and decreased absolute difference of both weight and height within the MZ pair were clearly observed from birth to the age of one. This features were more clearly seen in body height, suggesting that both weight and height in this period were genetically controlled and that this tendency was more apparently seen in height.

C 45

BLOOD PRESSURE IN TWINS. Toru KINOSHITA, Takeyasu IGARASHI, Minako FUJISHIMA, Akio ASAKA (Dept Health Sciences, Univ. Yamanashi Medical School, Yamanashi)

Blood pressures (BP) of twins, consisting of 97 MZ and 23 DZ, aged 13 to 46, were analyzed by the maximum-likelihood model fitting method to assess relative importance of the additive gene action (AG), the non-additive gene action (NG), the shared environmental effect (SE) and the non-shared environmental effect (NE). After adjusting age and sex, and then standardizing, the data were analyzed by LISREL7. The intraclass correlation coefficients of systolic BP were 0.58 and 0.29, for MZ and DZ, and those of diastolic BP were 0.09 and 0.03, respectively. The heritability using intraclass correlation coefficients was higher in systolic BP (0.54) than in diastolic BP (0.27). The best fitted model was as follows. The relative contributions of AG, NG and NE were 39%, 32% and 29% for systolic BP in the above order ($\chi^2=0.25, df=3, p=0.969$), and 9%, 32% and 59% for diastolic BP ($\chi^2=0.72, p=0.868$). These findings indicated that the gene action to BP may be explained not only by the additive genes but also by the non-additive gene(s), and that the relative influence of the non-shared environmental effect may be more important in diastolic BP than in systolic BP.

C 46

BASELINE AND EMS-INDUCED SCE FREQUENCY IN TWINS AND THEIR PARENTS. Tatsuya TAKESHITA, Toshimi OHMA, Zentaro YAMAGATA, Sumio IJIMA, Hitoshi HOSHINO, Akio ASAKA (Dept. Health Sci., Yamanashi Med. Col., Yamanashi)

Genetic and environmental factors on baseline and ethyl methane-sulfonate(EMS)-induced SCE frequency were investigated in 9 twin pairs (6 MZ, 2 DZ and 1 PZ) aged 12 years and their parents. Peripheral blood lymphocytes were added to the culture medium containing 40 μ M of BrdUrd and incubated for 70 hr. EMS (50 and 100 mM) was added at the start of the culture. Mean values of baseline SCE frequency in twins, fathers and mothers were 7.45 ± 0.22 , 9.97 ± 0.47 and 8.63 ± 0.35 , respectively. Each value was significantly different from the other two. Higher SCE frequencies in the parents would suggest a contribution of environmental factors such as smoking. Mean values of EMS-induced SCE frequencies were not different among the three groups. Intraclass correlation coefficients of baseline and EMS (50mM and 100mM)-induced SCE in twin pairs were -0.0125, -0.2603, 0.0382, respectively, and all were not significant. Therefore, contributions of genetic factors on baseline or EMS-induced SCE frequencies were not indicated.

C 47

SERUM IMMUNOGLOBULINS IN TWINS.

Zentaro YAMAGATA, Tatsuya TAKESHITA, Sumio IIJIMA, Takeyasu IGARASHI, Minako FUJISHIMA, Akio ASAKA (Dept. Health Sci. Yamanashi Med Col.)

The effect of genetic and environmental factors on serum immunoglobulin levels was studied in twins. Serum immunoglobulin levels were counted from 28 pairs (12-49 years old, MZ=23, DZ=5). The average of IgG level was 1311.2 ± 255.8 mg/dl (N=27) and the intraclass correlation coefficient of IgG level among MZ was 0.7439 ($p=0.0001$). Likewise, the averages and the intraclass correlation coefficients of IgA, IgM and IgE levels among MZ were 252.9 ± 101.7 mg/dl (N=27), 0.8351 ($p=0.0001$), 191.4 ± 69.5 mg/dl (N=27), 0.8594 ($p=0.0001$) and 122.6 ± 187.0 IU/ml (N=27), 0.4397 ($p=0.0022$), respectively, in the above order. For DZ, only IgG level ($r=0.7014$ $p=0.0248$) was significant. These results indicated that genetic factors may play an important role upon serum immunoglobulin levels. There were significant correlation coefficients of IgG and IgA levels between parents and children. The analysis by LISREL 7 indicated that environment factors were more important in serum IgE level than serum IgM level.

C 48

REGIONAL DIFFERENCE OF HLA HAPLOTYPE FREQUENCIES IN JAPANESE.

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The distribution of class I HLA haplotypes in 16 districts of Japan was examined. Using HLA types of unrelated blood donors, we have estimated haplotype frequencies of HLA-A and HLA-B loci for each local population. Although most HLA haplotypes were found in all of the local populations, considerable differences exist in their frequencies. For example, the frequency of A24-Bw52 haplotype exceeds 10% in Tokyo, Aichi, and Kyoto, but it is only 2% in Okinawa. A cluster analysis with respect to frequencies of prevailing HLA haplotypes yielded the following results. (1) In the Main Island of Japan, Kyoto, Aichi, Nagano, Tokyo, and Fukushima shared the most similar composition of HLA haplotypes. (2) South Kyushu and the Pacific coast of the Tohoku district belonged to the same cluster, which indicates close genetic relationship. (3) Okinawa considerably differed in composition of haplotypes from the other districts. Since the distribution of HLA haplotypes is affected mainly by migration events in the past, it provides valuable information on the origin and the history of the Japanese population.

C 49

Behavior of Blood Group Antigens on Red Blood Cells in Autoimmune Hemolytic Anemia.

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In patients with autoimmune hemolytic anemia (AIHA), red blood cell (RBC) blood group antigens were analyzed in detail. Several blood group antigens appeared to be acquired because they were not demonstrated hereditarily in the families. These antigens consisted of S in MNSs system, K and Kp^a in Kell-Cellano system. All antigenicities of the acquired antigens disappeared on the RBC membranes after the treatment with dispase or chymotrypsin. Therefore, all of the acquired antigens were considered to belong to glycophorin B-class blood groups. In AIHA there was another alteration of RBC blood groups. The reactivities of many RBC blood group antigens were lost when the hemolytic crisis was accelerated. The antigens were Le^a in Lewis system, c, D, E and e in Rh system, Fy^b in Duffy system, Jk^b in Kidd system and Xg^a in Xg system. Other blood group antigens also presented depression in the expression of the antigenic activities. The reactivities of these antigens were recovered after removing the sialic acid from patient's RBCs by neuraminidase treatment.

C 51**INTERSTITIAL DELETION OF CHROMOSOME 1p: 46,XX,del(1)(p32.3p34.1)**

Kyoko ABE, Naoki HARADA (Cytogenet. Res. Lab., Kyushu Med. Sci., Fukuoka), Kohichiro YOSHIURA, Norio NIKAWA (Nagasaki Univ. Sch. Med., Nagasaki), Makoto YOSHINO, and Shigeru KARUKAYA (Dept. Pediat., Kurume Univ. Sch. Med., Kurume)

A 28-day-old malformed girl with del(1p) was reported. She has a parietal cavernous hemangioma, short palpebral fissures, wide nasal alae, anteverted nostrils, a long philtrum, protruding upper labial commissure, low-set and malformed ears, bilateral nail hypoplasia of the second fingers, wide-set nipples, diastasis recti, long toes, and developmental retardation. Chromosome analysis was performed on her peripheral blood lymphocytes. High-resolution GTG-banding with the thymidine cell-synchronization method revealed a 46,XX,del(1)(p32.3p34.1) karyotype. C-like bands on chromosomes 1 obtained by AluI-banding revealed that the deleted chromosome 1 had been derived from her father.

There have been only 13 patients with 1p deletions. The deletion in our patient is smallest among the reported interstitial deletions for 1p. Since most of 1p shows light bands which are considered as GC-rich regions, few cases with 1p deletion may reflect the importance of these regions for development.

C 52

THE GENE LOCUS FOR TRANSIENT ABNORMAL MYELOPOIESIS (TAM) IS CONFINED TO 21cen-q22.2: CROSSING-OVER STUDIES OF 5 CASES

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We presented last year cytogenetic evidence for disomic homozygosity in trisomy 21 in 12 patients with TAM. We have confirmed this finding in the other 7 cases. Then, we proposed a hypothesis that a presumed recessive TAM-related gene must be located on chromosome 21. On the basis of the hypothesis, we studied crossing-over sites in the chromosomes 21 in 5 patients with both TAM and Down syndrome, who are informative for the parental origin (Table), using several probes as genetic markers (Fig. 1).

In every of the 5 patients, a segregation pattern of the p21-4U RFLP was consistent with the origin ascertained by previous cytogenetic studies. However, in 2 patients, the pGSE8 RFLP segregation was inconsistent with the cytogenetic data (Table, Fig. 2). These findings may indicate that the presumed TAM gene is located between centromere and locus D21S17.

C 53

THE GENE LOCI FOR ANGELMAN SYNDROME AND PRADER-WILLI SYNDROME ARE DIFFERENT

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Since Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are often associated with del(15)(q11.1 q12), an identical gene locus for the 2 syndromes has been hypothesized. To test this, we studied 13 AS and 52 PWS patients on the ranges of their DNA deletions.

Eight (61.5%) of 13 AS patients, and 32 (61.5%) of 52 patients showed deletions in one or more of the 5 loci examined. However, deletion ranges were different between the two. A common deletion site among AS patients is confined to a segment between loci D15S11 and D15S10, whereas the site among PWS to a segment between D15S9 and D15S12. Since the five loci examined are ordered as cen-D15S18-D15S9-D15S12-D15S11-D15S10, the findings suggest that the genes responsible for the two syndromes are different. On the basis of the results, we propose a hypothetical model for the occurrence of PWS, explaining all the reported abnormal karyotypes in PWS patients. In case of AS, the mechanism is contrary.

C 54

GENOMIC IMPRINTING IN ANGELMAN SYNDROME (AS) GENE LOCUS

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AS is often associated with chromosome microdeletion at 15q11-q12, but its role in the occurrence of AS remains unclear. AS and Prader-Willi syndrome (PWS) show similar cytological deletions but the opposite parental origin of deletion: maternal origin in AS vs. paternal origin in PWS. Thus, the "genomic imprinting" hypothesis has been proposed that parental genes at 15q11-q12 are differently imprinted and gene behaviors are different in offspring: a loss of a maternal allele may lead to AS, while a loss of a paternal allele may result in PWS. In this model, an alternative phenotype of AS is PWS.

RFLP study on an AS family revealed that all the 3 affected children have deletions at D15S10 locus, and both of the phenotypically normal mother and a maternal grandfather also have deletions at this locus. This finding supports the genomic imprinting event that an individual who inherits a deletion from mother has AS phenotype, but does not support the model that PWS is the alternative phenotype of AS.

C 55

SMITH-MAGENIS SYNDROME IN TWO JAPANESE BOYS; INTERSTITIAL DELETION OF THE SHORT ARM OF CHROMOSOME 17. I.KONDO(DEPT. OF HUM ECOL AND GENET, UNIV. OF THE RYUKYUS, OKINAWA), K.KUWAJIMA(DEPT. OF PEDIATR, IBARAKI HANDICAP CHILD. HOSP) and N.NIKAWA(DEPT. OF GENET, UNIV OF NAGASAKI, NAGASAKI).

We describe two Japanese male patients with interstitial deletion of the short arm of chromosome 17, del(17)(p11.2p11.2). They aged 8 and 17 years, had typical clinical manifestations of the Smith-Magenis syndrome, including psychomotor and growth retardation, brachcephaly, midface hypoplasia, everted upper lip, prognathism, short broad hand and fingers and behavior problems. Characteristic behavior problems such as hyperactive and self-mutilation are the most important for the diagnosis of this syndrome and chromosome analysis with specific attention to 17 is necessary to detect this deletion.

C 56

LINKAGE STUDY OF AUTOSOMAL DOMINANT SPINOCEREBELLAR ATAXIA. Akemi WAKISAKA, Hidenao SASAKI, Kunio TASHIRO, Akio TAKADA (Dept. Pathol. Neurol. Hokkaido Univ.), Michihiro C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ.) Takeshi HAMADA (Hokuyukai Neurol. Hosp.)

Linkage study was performed for 17 pedigree of dominant olivopontocerebellar atrophy(OPCA), 17 pedigrees of Joseph disease, and 5 pedigrees of dominant cortical cerebellar atrophy("Holmes' ataxia") to 57 polymorphic markers, including 23 markers on chr. 6. Lod scores were computed by using LIPED program with age at onset correction. However, none of them showed close linkage to those disease loci. Other genetic markers also did not linked to any of three disorders. These results indicate that Joseph disease is genetically different from SCA1 (HLA-linked OPCA). It leaves unknown whether heterogeneity might exist even within dominant OPCA. Further studies as well as making the fine linkage maps on 6p chromosome by using multi-point linkage analysis are necessary to dissolve this problem.

C 57

CONSTRUCTION OF A COSMID LIBRARY CONTAINING HUMAN CHROMOSOME Xq24-Xqter. Kiyotoshi KANEKO, Tadashi MIYATAKE and Shoji TSUJI. Department of Neurology, Brain Research Institute, Niigata University

Adrenoleukodystrophy (ALD) is an X-linked recessive neurologic disorder, whose chromosomal locus has been mapped to Xq28. To identify the molecular defect of ALD, we have constructed a cosmid library using a human-hamster cell hybrid, X3000-11.1 (kindly provided by Dr. Nussbaum) carrying human Xq24-Xqter. High molecular weight genomic DNA extracted from X3000-11.1 are partially digested with Sau3AI, size fractionated, partially filled-in and dephosphorylated. The genomic DNA was ligated to XhoI digested and partially filled pWE15 cosmid vector. We obtained 100,000 colonies per 1 µg of human genomic DNA. The average size of inserts were 30.2 to 40.9 Kbp (mean; 35.3 Kbp, SD; 4.0). 118 colonies out of 50,000 colonies (0.24%) hybridized to total human genomic DNA, and we confirmed that randomly picked 4 clones out of 118 clones hybridized to human X chromosome. A clone with an internal NotI site was isolated, and now we are screening as many as 1,500,000 colonies to isolate 3,000 cosmid clones (2 genome units) containing human Xq24-Xqter region.

C 58

FACTORS AFFECTING EXPRESSION OF A COMMON FRAGILE SITE, fra(3)(p14).
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Cancer Center, Chiba), Ei-ichi TAKAHASHI, Hideo TSUJI and Tada-aki
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We have been studying inter-individual variation in expressivity of a common fragile site, fra(3)(p14), in both populations of healthy blood donors and patients of various diseases. Chromosome test was done by 72hr whole blood culture with folate deprivation (M-F10) for 1629 subjects, and aphidicolin treatment (APC) for 294 subjects. Furthermore, cell proliferation rate of cultured lymphocytes was examined for 149 subjects by using differential staining of sister chromatids. For only 32 healthy subjects, folate concentration in blood plasma was examined. Expression of fra(3)(p14) with M-F10 was more predominant in male than in female, and in younger than in older subjects. It also showed a seasonal variation, being maximized in late autumn and minimized in summer. Similar variations were also observed in the APC induced expression. Those cancer patients with unfavorable prognosis showed a higher mean expressivity than the other patients. Cell proliferation rate, though it also exhibited a similar pattern of sex and age differences, was not correlated with the expressivity of fra(3)(p14), whereas folate concentration in blood plasma showed a negative correlation with the latter.

C 59

CHANGES OF CHROMOSOMAL FRAGILITY BY THE MENSTRUAL CYCLE: Hisako OCHI¹,
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Changes of number and sites in spontaneous and aphidicolin(APC)-induced breaks on chromosomes and frequencies of sister chromatid exchanges(SCE) from peripheral lymphocytes during the menstrual cycle were examined in eight healthy women. Menstrual cycle was determined by the menstruation and the quantity of serum luteinizing hormone, follicle-stimulating hormone, estradiol and progesterone. The number of spontaneous breaks at the follicular, the interval including ovulation and the luteal phases were 0.03 ± 0.01 , 0.03 ± 0.02 and 0.04 ± 0.03 /cell, respectively. The number of APC($0.2 \mu\text{M}$)-induced breaks were 0.96 ± 0.23 , 0.91 ± 0.14 and 1.23 ± 0.20 /cell, respectively. The higher frequency at the luteal phase was statistically significant. In the luteal phase, breaks at bands 2q32, 3q27, 6q26 and 16q23 increased and breaks at band 9q32 decreased significantly. Frequency of SCE at the luteal phase showed higher than other phases, but no significance was seen.

C 60

GENETIC LINKAGE STUDY ON AFFECTIVE DISORDERS -SECOND REPORT-. Shinichiro NANKO¹, Masaru KOBAYASHI², Noboru TAKAZAWA¹, Hajime KAZAMATSURI¹, Shinobu GAMOU³, Jun KUDOH³, Nobuyoshi SHIMIZU³, Toshiyuki FURUSHO⁴ (¹Dept. Psychiat., Teikyo Univ., ²Genetic Lab., SRL Inc., ³Dept. Mole.Biol., Keio Univ., ⁴Dept. Clin. Genet., Kyorin Univ., Tokyo)

We have investigated two pedigrees in an attempt to detect the putative linkages between affective disorders and c-Ha-ras-1 oncogene (HRAS1) and insulin gene (INS) on chromosome 11 as well as between hypoxanthine phosphoribosyltransferase (HPRT) on X chromosome. Subjects were 17 members of two families including 6 individuals with affective disorders. The diagnoses were based on the Research Diagnostic Criteria of Spitzer. DNA samples from members of the pedigree were digested with the restriction enzymes SacI and BamHI. Hybridization was carried out with DNA probes HRAS1 and INS to SacI digests and HPRT to BamHI digests. The linkages for HRAS1 and INS probes were ruled out in both families. The linkage for HPRT was ruled out in one family and X-linked transmission was obviously ruled out by the form of inheritance in the other family. Thus, the linkages between affective disorders and chromosome 11 and X were ruled out in these families. This would indicate that the gene or genes responsible for affective disorder in these families are at other, as yet unknown, loci if ever.

C 61

AUTOMATED ANALYSIS OF SERUM α -L-FUCOSIDASE (FUCA2). Ryozo KASAI¹, Kouji NARAHARA², Yuji YOKOYAMA², Kazushiro TSUJI², Masae MURAKAMI² and Yoshiki SEINO² (¹Asahigawa Jidoin Hosp., Okayama and ²Dept. Pediatrics, Okayama Univ., Okayama)

Serum α -L-fucosidase (FUCA2) is an important genetic marker. We described a simple and rapid method for measurement of FUCA2 activity by using an automatic analyzer.

The assay was based on the method of Zielke et al.(1972) with minor modification. Sera from 132 normal subjects were measured by the automatic analyzer (Hitachi Ltd., Model 7050) as follows: 20 μ L of samples were incubated with 120 μ L of sub-strate consisting of 2 mM p-nitrophenyl (PNP)- α -L-fucoside in citrate-phosphate buffer (0.05 M, pH 5.0) for 4.7 minutes at 37°C. The reaction was terminated and colored by adding 120 μ L carbonate buffer (0.25 M, pH 10.15). The released PNP was measured at 415 and 505 nm. Precision studies yielded CV's of 3.9% in intra-assay (N=10) and 6.8% in inter-assay over 7 assay runs. FUCA2 activities from 132 subjects gave a mean value of 8.42 IU/L and SD of 2.55 IU/L and ranged from 3.5 IU/L to 16.2 IU/L. The assay time is about 30 min for 50 samples. There is a significant correlation between activities by this method and those by the fluorometric method (Wood, 1975) (N= 57, $\gamma=0.81$, $P<0.001$).

The results indicate that this method is simple, rapid and reproducible for measuring FUCA2 activities. It is also suitable for large-scale screening for searching FUCA2 activity variants.

C 62

STUDY ON A HP CARLBERG VARIANT WHICH OCCURRED AS A RESULT OF QUANTITATIVE UNBALANCE OF HP GENE PRODUCT. Jun-ichi ASAKAWA, Mieko KODAIRA, Eiko NISHIKORI, Satomichi KANEOKA, Junko KANEKO, Chiyoko SATOH. (Dept. of Genet., RERF, Hiroshima)

The plasma haptoglobin (HP) of 23,326 Japanese children was examined by electrophoresis. The variants found in four children were very similar to the HP Carlberg (CA) variant, and exhibited an electrophoretic pattern similar to that produced from a mixture of HP 1-2 and HP 2. The molar ratio of HP α 1 to HP α 2 polypeptide of the variants was about 1/3. Among the four variants, two were observed only in the children. Previously, we proved that the two children were mosaic for HP 1-2 and HP 2-2 cells. Furthermore, the mosaicism originated from a reverse mutation from HP*2 to HP*1. In this presentation we report on the third CA variant which was detected in both child and mother. Southern blot analysis of the DNA from single colonies revealed that all colonies, 35 from the child and 27 from the mother, showed the HP*1/HP*2 genotype. No mosaicism was detected. Thus, the variant was thought to originate from an abnormal expression of the variant HP gene inherited from the mother. Analyses of DNA from the variant detected no deletions or rearrangements in the HP gene or the 3.4 kb 5' flanking region when analyzed by southern blotting. Sequence analysis of the 5' flanking region of the variant HP gene is under study.

C 63

FETAL SEXING ON CHORIONIC VILLUS CELLS AND AMNIOCYTES USING DNA AMPLIFICATION. Tadashi SUMI, Ritsuko ADACHI, Tadashi IIDA, Setsuo OKADA, Kaoru SUZUMORI, Yoshiaki YAGAMI. Dept. Obstet. and Gynecol., Nagoya City Univ. Med. Sch., Aichi.

The polymerase chain reaction(PCR) is a method for selective amplification of DNA segments. In the present study, we have attempted the fetal sexing on chorionic villus cells and amniocytes by conventional cytogenetic analysis and by the presence of a Y-specific DNA sequence in the cells using PCR. Four chorionic villus samples and forty six amniocytes were analyzed. The results of the cytogenetic tests and PCR analyses were compared only at the end of investigation. There was a good concordance between the results of sexing by cytogenetic method and by detecting the presence of a Y-specific DNA in the cells. In addition, we have tried to amplify a X-Y homologous sequence by PCR for fetal sexing. However, X-Y homologous sequence was not amplified. We thought that further improvement of this procedure would be necessitated. We concluded that PCR gene amplification would be an efficient and rapid method for the prenatal diagnosis of hereditary disorders in near future.

C 64

ULTRAVIOLET PHOTOGRAPHY AS A DIAGNOSTIC MEASURE OF HYPOMELANOSIS OF ITO AND DERMATOGLYPHIC PATTERNS. Hirofumi OHASHI, Ichiro MURANO, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

An Asahi Pentax single reflex camera equipped with an Ultrachromatic Takumar lens was used in combination with a Nikon flash with a 360 nm bandpass filter. A Fuji Neopan SS (ASA 100) film was used and developed with Fuji Microfine. The ultraviolet photography increased the contrast of the disorders of pigmentation of the epiderm, including hypomelanosis of Ito. It also increased the resolution of the dermatoglyphic patterns.

C 65

FLUORESCENCE *IN SITU* HYBRIDIZATION WITH CHROMOSOME SPECIFIC PROBES. MASATO Tsukahara, Ichiro MURANO, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube), Kohsuke SASAKI (Dept. Pathology, Iwate Med. Univ., Iwate), Joe W. Gray (Biomedical Science Division, Lawrence Livermore National Laboratory)

Fluorescence *in situ* hybridization was performed using flow cytometer-sorted chromosome-specific probes in chromosome preparations of trisomy 21(47,XY,+21) and an 8/9 translocation(46,XY,der(9),rec(8;9)(q22;q24)). Chromosome 21 specific DNA probes were labeled with biotin and detected with FITC. Chromosome 8- and 9-specific DNA probes were labeled respectively with biotin and AAF(N-acetoxy-2-aminoacetylfluorene). Chromosome 8-specific DNA sequences were detected with Texas red, while chromosome 9-specific DNA sequences were detected with FITC. In metaphase spreads, 21 trisomy and 8/9 translocation were easily determined. The fluorescence *in situ* hybridization method is useful in the diagnosis of both numerical and structural chromosomal abnormalities.

C 66

MOLECULAR CYTOGENETIC STUDIES OF BONE AND SOFT TISSUE TUMORS: Xin MAO¹, Syuiti ABE¹, Takayuki NOJIMA², and Michihiro C. YOSHIDA¹ (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ. and ²Dept. Pathol., Hokkaido Univ. Sch. Med., Sapporo)

Seeking for the possible genetic mechanism involved in the development of bone and soft tissue tumors, we have performed molecular cytogenetic analyses on 13 rhabdomyosarcomas (RMS), eight malignant fibrous histiocytomas (MFH), three Ewing sarcomas (ES), and one synovial sarcoma (SS). Among them, several cases exhibited some consistent chromosome abnormalities such as t(2;13)(q37;q14) in alveolar RMS, t(11;22)(q24;q12) in ES and t(X;18)(p11;q11) in SS. However, no consistent chromosome abnormality was found in MFH cases examined here. Southern blot analysis revealed RFLPs in some gene loci including those localized at or near the breakpoints of the above consistent chromosome abnormalities. In particular, RFLPs at VNTR loci localized to 1p (D1S57), 2p (D2S44) and 17p (D17S30) were frequently observed in most of the tumor cases examined. The present findings suggest that a wide spectrum of genomic instability may be involved in the pathogenesis of bone and soft tissue tumors.

C 67

TRANSFORMATION OF FIBROBLASTS FROM COMPLETE MOLES WITH ori⁻ SV40 DNA

Ichiro MURANO, Shinya MATSUURA, Kiyosato MATSUO, Tadashi KAJI (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

XX complete moles usually originate from duplication of a haploid sperm in an empty ovum, and thus are completely homozygous. XY moles arise from dispermy, and so are 50% homozygous and 50% heterozygous. Immortalized, completely homozygous cells would be useful for various genetic studies. Transformation of cultured molar fibroblasts was attempted by transfecting two XX moles and one XY mole with ori⁻ SV40 DNA. The transfected molar fibroblasts changed their morphology, grew vigorously for 23 to 33 passages, and then died out. An additional trick is apparently needed to immortalize molar fibroblasts.

C 68

CHROMOSOME BANDING BY RESTRICTION ENZYMES: Xin MAO, Syuiti ABE and Michihiro C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Inducibility of chromosome bands by AluI, HaeIII and Sau3AI restriction enzymes (REs) was compared on routinely processed human chromosome preparations. These REs, each recognizing different four basepair cleavage site, could induce G- and/or C-bands. AluI digestion produced clear G- and C-bands on most members of the chromosome complement. On the other hand, HaeIII and Sau3AI digestion resulted in C-band, rather than G-band, prominently on chromosomes 1, 9, 16, and Y. Enzyme concentration had some effects on the yields of both bands, but aging of chromosome slides did not seem to have a significant effect. Interestingly, AluI buffer itself, with low salt concentration, was capable of producing similar banding patterns to those induced by the enzyme, although HaeIII and Sau3AI buffers with middle and high salt concentration, respectively, hardly exerted such effects. Our findings suggest that RE banding may be related to the specific DNA sequences in chromosomes, and that RE buffer composition such as salt concentration may play a role in producing RE bands, in addition to enzyme digestion.

C 69

CARDIO-FACIO-CUTANEOUS(CFC) SYNDROME: REPORT OF 3 PATIENTS WITHOUT HYPERKERATOTIC LESIONS.

Ichiro MURANO, Yukihisa MATSUDA, Osamu KONDOH, Kiyosato MATSUO, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Cardio-facio-cutaneous syndrome is a recently described syndrome of mental and growth retardation, relative macrocephaly, unusual facies, webbed neck, sparse hair, hyperkeratosis of the skin, and congenital cardiac defect (Reynolds et al. 1986). Three boys with the syndrome are reported, ranging in age from 3 to 9 years. These boys showed most of the clinical manifestations of the CFC syndrome, but lacked hyperkeratotic skin lesions. Their cardiac defects were: hypertrophic cardiomyopathy; valvular pulmonary stenosis and atrial septal defect; and supra-valvular aortic stenosis.

C 70

GENES AND ENVIRONMENTAL FACTORS IN ALZHEIMER'S DISEASE.

Kiyotaro KONDO (Dept. Publ. Hlth, Hokkaido Univ. Sapporo, Japan)

Alzheimer's disease is a degenerative disease of the brain causing progressive dementia. It is probably heterogeneous involving at least ; 1) autosomal dominant cases with presenile onset in which genes on the chromosome 21 or 19 code the amyloids, and 2) senile cases usually occurring sporadically, but sometimes familial. The former is few (5 - 6 % or less ?). No formal-genetic analysis is not made, however. Heritability was about 80 % based on the twin data .

Case-control studies disclosed 1) head trauma, 2) loss of teeth, 3) low school education, 4) inactive psychosocial behaviors 15 - 20 years prior to AD , and low physical activities since young ages, as the putative risk factors.

It may be that, few Mendelian cases aside, AD is a final consequence of a complex interaction of genes and enviromental factors. Further studies are necessary to solve the nature / nurture problem in this disease.

C 72

HETEROGENEITY OF TYPICAL RETINITIS PIGMENTOSA IN JAPAN

Keiko Fujiki, Mutsuko Hayakawa, Yoshihiro Hotta, Utako Tanabe, Atsushi Kanai and Akira Nakajima (Juntendo University, Tokyo, Japan)

Typical retinitis pigmentosa (RP) is a group of disorders of the retina leading to blindness with multiple genetic types ; autosomal recessive, autosomal dominant and X-linked. The purpose of the present study is to evaluate the proportion of the different types of transmission of RP, especially X-linked in Japanese population.

A total of 385 patients (220 males and 165 females) from 379 families with RP (43% from Tokyo, 35% from surrounding prefectures and the remains were from other part of Japan) were obtained at Department of Ophthalmology, Juntendo University Hospital in a period from 1980 to 1988. We analyzed these data by inbreeding analysis using the proportion of consanguineous marriages of parents, segregation analysis using the distribution of affected siblings and the pattern of transmission in pedigree. The proportion of an autosomal recessive was estimated to be 32-39% ; autosomal dominant, 11-12% ; sporadic or isolated cases, 42-51% ; and X-linked, approximately 5%. About 3% of simplex cases without consanguinity had an affected child.

C 73

A CASE OF MITOCHONDRIAL ENCEPHALOMYOPATHY WITH THE FEATURES OF ATYPICAL PSYCHOSIS. Yukari UEDA, Naoyasu MOTOMURA, Yasuhiro NONOMURA, Katsuhiro TOYODA, Hiroshi YONEDA, Toshiaki SAKAI, Daisuke FURUDAMA*, Keiichi SHINODA*, Nakaaki OSAWA* (Dept. Neuropsychiatry, 1st Department of Internal Medicine*, Osaka Medical College, Takatsuki)

Mitochondrial encephalomyopathy (ME) is characterized by muscular atrophy, heart block and dementia, and is transmitted by maternal inheritance. We report here a case of ME exhibiting symptoms of atypical psychosis.

The patient is a 29 years old female who has had tonic-clonic convulsions since 23 years of age. She developed a hallucinatory-delusional state with disturbed consciousness and psychomotor excitement after the 5th attack of convulsions. Plasma levels of lactate and pyruvate were high. Low signal intensity areas in the basal ganglia were demonstrated by MRI. Muscular biopsy showed ragged red fibers. None of her relatives showed ME symptoms. However the CT of her brother showed high density areas in the basal ganglia, suggesting that he is a genetic carrier. CT and MRI may be a useful tool for detecting genetic carriers of ME. There have been only a few case reports of ME with psychotic symptoms. However we consider the psychotic features as clinical manifestations of ME.

C 74

TWO CASES OF TURNER'S SYNDROME WITH HALLUCINATORY-DELUSIONAL STATE. Yoshihiro KONO, Toru ISHIDA, Yukari UEDA, Hiroshi YONEDA, Katsuhiro TOYODA, Yasuhiro NONOMURA, Toshiaki SAKAI (Dept. Neuropsychiatry, Osaka Medical College, Takatsuki, Osaka)

Turner's syndrome accompanied by psychotic symptoms is rare and only 5 such cases have been reported in Japan. We present 2 cases with karyotype 45XO, exhibiting hallucinatory-delusional symptoms. Case 1 is most probably complicated with schizophrenia, consideration being given to the age of onset, hereditary factors and the course of illness. The psychotic symptoms of case 2 are probably related to mental retardation (IQ 46). In these 2 cases the causes of psychotic symptoms are seemingly different. Pathogenesis of the psychotic manifestation in Turner's syndrome is most probably unrelated to sex chromosome.

C 75

LINKAGE ANALYSIS OF HYPERTROPHIC CARDIOMYOPATHY

Hirofumi NISHI¹⁾²⁾, Akinori KIMURA¹⁾, Kohmei MATSUYAMA²⁾, Yoshinori KOGA²⁾, Hironori TOSHIMA²⁾ and Takehiko SASAZUKI¹⁾ (¹⁾Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka, ²⁾3rd Dept. Int. Med., Kurume Univ., Kurume, Japan)

Hypertrophic cardiomyopathy(HCM) is a myocardial disease with unknown etiology. The majority of patients show familial occurrence consistent with autosomal dominant inheritance. One approach which can be used to identify the causative gene would be to demonstrate genetic linkage between HCM and a genetic marker. In this study, we analysed 117 members from 29 multiplex families with HCM (70 affected and 47 unaffected individuals), using 50 genetic markers. Among the probes tested, 12 probes showed no linkage to the locus for HCM. One of the genetic markers tested, maximum lod score ($\hat{z}$) 3.18 was obtained by using PALB(pHH64) as a marker ($\theta=0.00$, penetrance was set to 90%). The observation suggested the linkage between major gene for HCM and PALB in Japanese.

C 76

LINKAGE ANALYSIS OF THE FRAGILE X SYNDROME. I.KONDO, M.YONAHARA (DEPT OF HUM ECOL AND GENET, UNIV OF THE RYUKYUS, OKINAWA), K.SUZUMORI, R.ADACHI (DEPT OF GYNEC, NAGOYA CITY UNIV, NAGOYA), N.NIIKAWA, K.TSUKAMOTO (DEPT OF GENET, NAGASAKI UNIV, NAGASAKI), T.KAJII AND S.KUWANO (DEPT OF PEDIATR, YAMAGUCHI UNIV, YAMAGUCHI)

The fragile X syndrome has been established as the most common familial form of mental retardation. Despite extensive study, the relationship between the disease and the fragile site of X chromosome is still unclear and cytogenetic diagnosis is sometimes difficult in patients with mental retardation and in carrier females. Therefore, DNA analysis using DNA probes flanking marker at Xq27.3 is helpful to definitive diagnosis of the fragile X syndrome. In 10 families with the fragile X syndrome, we have studied linkage using two DNA probes (pRN1 and VK21C), nearest flanking markers at both sides of the fragile site in Caucasian families. DNA probe, pRN1 detected Xmn I polymorphism in a Japanese population, but VK21C did not show MspI polymorphism. In 6 informative families, a genetic distance between the fragile X syndrome and pRN1 locus(DXS369) is about 5 cM at Lod score 2.811 in Japanese families.

C 77

LINKAGE ANALYSIS OF MEN 2A USING THE *ret* PROTO-ONCOGENE

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Yukihito ISHIZAKA³, Takesada MORI¹, and Shin-ichiro TAKAI⁴ (¹2nd Dept.
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 Osaka Univ. Med. Sch., Osaka. ³Natl. Cancer Cent. Res. Inst., Tokyo)

The gene responsible for multiple endocrine neoplasia type 2A (MEN 2A) has been mapped to the pericentromeric region of chromosome 10. In order to clarify the genetic abnormalities of MEN 2A, the analysis of many samples using flanking DNA markers should be required. Recently, the *ret* proto-oncogene, one of the receptor-tyrosine kinases has been mapped to 10q11.2. We tried to perform the linkage study using the *ret* proto-oncogene (*c-ret-2*). Seven out of 14 Japanese MEN 2A families were informative, and the maximum lod score was 4.62 at the recombination rate of 0.00. This result indicates that the *ret* proto-oncogene is useful not only for the prediction of gene carrier, but also for the isolation of the candidate gene of MEN 2A itself.

C 78

A CASE OF OSTEODYSPLASTIC PRIMORDIAL DWARFISM, TYPE I OR TYPE III.

A. GOSHIMA^{1,3}, Y. HAYASHI¹, D. KATSUMATA¹, R. NAGOSHI¹, Y. TORIYAMA¹, S. OHDE¹
T. OHNO¹, G. NISHIMURA⁴, Y. FUKUSHIMA² (Division of Neonatology¹ and Medical
 Genetics², Saitama Children's Medical Center, National Defence Medical
 College³, Shimizu City Hospital⁴)

The probanda, a female newborn, was born at 39w5d to 33y father and 28y mother, both healthy and nonconsanguineous. A 1y brother was healthy. The pregnancy was complicated with IUGR and diminished fetal movement. The birth weight was 1,126 g. At birth, the following anomalies were noticed: marked growth retardation, microcephaly, sparse scalp hair, exophthalmos, a large nose, micrognathia, thin and hypopigmented skin, hypoplastic nipples and hypoplastic labia major. The bone X-ray films showed hypogenesis of pelvis, elongated clavicle, broad metaphysis and bowing of long bones. The brain CT revealed agenesis of corpus callosum. The heart, kidneys, GI tract and karyotype were normal. These findings were compatible with osteodysplastic primordial dwarfism (OPD) type I or type III. OPD I and OPD III may be a similar disease and the difference could be originated from the age the patient was diagnosed. This is the 1st case in Japan and the 6th in the world.

C 79

PHENOTYPE MAPPING OF THE LONG ARM OF CHROMOSOME 4. Keiko WAKUI¹, Hiroyoshi IMURA¹, Haruo MATSUMOTO¹, Toshiro NISHIDA¹, Yoshifumi YAMAMOTO³, Yoshimitsu FUKUSHIMA² (¹Dept. Clinical Lab., ²Div. Med. Genet., Saitama Children's Medical Center, and ³Dept. Pediatr., Tokyo Metropolitan Police Hospital)

We reported 3 partial monosomies and 3 partial trisomies of the long arm of chromosome 4 [①46,XY,del(4)(q12q21.1), ②46,XY,del(4)(q13q23), ③46,XX,del(4)(q27q28.2), ④46,XY,-11,+dir ins(11;4)(q25;q21.1q27)mat, ⑤ 46,XY,-3,+der(3),t(3;4)(q29;q27)de novo, ⑥46,XY,-15,+der(15),t(4;15)(q31;q11.2)mat].

On the basis of clinical and cytogenetic findings of the present cases and those of patients reported in the literature, we tried to establish the phenotype map of 4q. The following relations are noted: del(4q12-q21) and piebald trait, del(4q27-q32) and hypoplastic nails, del(4q31-qter) and 4q terminal deletion syndrome characterized by MR, short nose, cleft lip /palate, displacement of toes, syndactyly and CHD, dup(4q28-q31) and renal anomaly, dup(4q31-qter) and 4q terminal trisomy syndrome consisting of MR, microcephaly, large and low-set ears, narrow palpebral fissures, ptosis, carp mouth, cryptorchidism, CHD, renal anomalies, and feet malformations.

C 80

A CASE OF PARTIAL TRISOMY 4q: Toshiyuki IGUCHI, Yasuhiko ITO, Masami ASAI, Jin ITO, Noboru OKADA (Pediatr. Yokkaichi Municipal Hospital, Mie), Hidetsune OISHI (Gen. Insti. Aichi Prefectural Colony, Aichi)

症例は日齢0の女児で、父34才母30才で患児は第2子。第1子は正常。在胎39週3日2810g, 1分Ap 8点, 頭位自然分娩。生後ときどき呼吸停止チアノーゼを認め入院。身長47cm, 頭囲31cm(小頭症), 色素がうすく粗い毛髪, 小さい口, 尖頭状高口蓋, 耳介低位付着, 耳介聳立, 第5指内彎, 両側くも指, 第4指 overlapping⊕ 深い仙骨部小窩。心不全, 腎不全, 強直発作(てんかん)を認めた。生後1ヶ月肥厚性幽門狭窄症にてRamstedt手術施行。生後10ヶ月 身長54.5cm, 体重2865g 経管栄養中。定頸(+), 追視(+), 寝返り(-), 坐位(-)。

生後11ヶ月家庭にて呼吸停止し死亡。剖検にて, 心奇形は大動脈離断, 心室中隔欠損症, 腎奇形は左低形成腎, 両腎とも尿酸塩性萎縮腎となっていた。

細胞遺伝学的検索にて, 患児は, 46, XX, dir dup(4)(q 21.1→q 31.2), 父母ともに染色体は正常であった。

C 81

A CASE OF 6q DISTAL MONOSOMY. Yuko ENDO, Atsushi KOMIYAMA (Dept. Pediatr., Shinshu Univ. Sch. Med., Matsumoto) Yoshio YAGI, Yoshimi ASAGAI (Shinano Medical Welfare Center, Shimosuwa) and Kouji NARAHARA (Dept. Pediatr., Okayama Univ. Sch. Med., Okayama)

The patient is a 17-year-old male. After 9 months of gestation, he was born by cesarean section weighing 2760 gr. His father was 34 years old and his mother 33, on his birth. He needed tube feeding because of the cleft palate. He showed severe developmental delay; he has been institutionalized. His body height is 115.8 cm, body weight 26.8kg and head circumference 50cm, all below 3% tile. His IQ is 26. He has microcephaly, flat occiput, hypertelorism, epicanthus, strabismus, upslanting palpebral fissures, large and malformed ears, preauricular pit, long philtrum, short neck, low posterior hair line, severe kyphoscoliosis, congenital dislocation of the hip joints, flat feet, pes valgus, cleft palate, slight cataract and hearing difficulties. On dermatoglyphics, from I to V, U, A, U, U, U, (left), A, U, A, U, A, (right); MLI are 12, 12 and TRC is 34. His karyotype using high resolution banding method is 46,XY,del(6)(q25.1). His PLG level is $3.6 \frac{\mu\text{g}}{\text{mg}}$ (normal range 10.8 ± 1.8) and 43% (normal range 105 ± 14) and FUCA₁ level is $11.3 \frac{\mu\text{mol}}{\text{min}} / \frac{\text{mg}}{\text{protein}}$ (normal range 9.5 ± 2.1).

C 82

ALAGILLE SYNDROME (ARTERIOHEPATIC DYSPLASIA) AND 20p PARTIAL MONOSOMY. Y. FUKUSHIMA¹, Y. MURAMATSU², S. IMAIZUMI³, Y. UEOKA⁴, K. OGAWA⁵, J. NISHIMURA⁶, S. YAMAGUCHI⁷ (Division of Med. Genet.¹, Nephrol.², Surg.³, Ophthalmol.⁴, Cardiol.⁵, Plast. Surg.⁶, Metabol.⁷, Saitama Children's Medical Center)

Alagille syndrome is a malformation syndrome characterized by five major features: intrahepatic biliary hypoplasia, peculiar facies, CHD (PS), vertebral anomaly and posterior embryotoxon. We reported 2 cases with this syndrome. In addition to major features, Case 1 (2m, male) had renal hypertension and Case 2 (2y, female) had cleft palate, and severe growth and mental retardation. High-resolution chromosome analysis revealed a normal karyotype in case 1 and 20p partial monosomy [46,XX,del(20)(p11.2)] in Case 2. Case 2 is the 5th patient with both Alagille syndrome and 20p-. The Alagille syndrome patients with 20p- seemed to be susceptible to growth and mental retardation and cleft palate, other than major features. Alagille syndrome is likely to be one of the contiguous gene syndromes located at 20p.

C 83

A CASE WITH A 46,XY,rec(11),dup p,inv(11)(p13q24.2)mat KARYOTYPE. Hiromi SAKAMOTO, Jun-ichi FURUYAMA, Osamu MIKAMI, Masafumi HANDA, Yoshie SUGAWARA, Hiroko MINURA (Dept. Genet. and Clin. Genet., Hyogo Col. of Med., Nishinomiya) and Ryoichi KOGA (Dept. of Neonat., Osaka Med. Cen. and Res. Inst. for Mat. and Child Health, Izumi).

A boy was born on June 14, 1989 to the first child of 30-year-old father and 27-year-old mother. Mother had no history of miscarriage. At 27-week-gestation, developmental delay and decrease in amniotic fluid were noticed and referred to Osaka Medical Center. At 30-week-gestation, he was born by Caesarean section. At birth, his weight was 634 gr (-3.3 SD), his height 31.0 cm (-3.3 SD), his head circumference 25.0 cm (-1.5 SD), Apgar score was 1. He developed severe gas exchange insufficiency and was supported by respirator. He also showed hyperhirsutism, low hair line, low set ear, hypertelorism. After cytogenetical study, his chromosome 11 was revealed to be abnormal. Father's karyotype was normal, but Mother had a pericentric inversion in chromosome 11. We decided his karyotype as 46,XY,rec(11),dup p,inv(11)(p13q24.2)mat. He was a trisomy of 11pter→p13 and a monosomy of q24.2→qter. He was fed about 100 Cal/Kg/day, but his body weight did not increase well. At the day of 168 after his birth, he died and weighted only 1188 gr.

C 84

RUBINSTEIN-TAYBI SYNDROME IN A CHILD WITH A DE NOVO RECIPROCAL TRANSLOCATION,t(2;16)(p13.3;p13.3). Kiyoshi IMAIZUMI,Yoshikazu KUROKI, Div. Med. Genet. Kanagawa Children's Med. Cent. Mutsukawa 2-138-4, Minamiku Yokohama.

We report a female patient with both the typical Rubinstein-Taybi syndrome and a balanced reciprocal translocation between chromosome 2 and 16. The patient had some clinical manifestations with the RTS, such as mental retardation, growth retardation, downward slanting palpebral fissures, beaked nose with nasal septum extended below alae nasi, hypoplastic maxilla with narrow palate, broad thumbs and big toes. High resolution chromosome analysis of the patient revealed a reciprocal translocation without visible chromosomal deletions, karyotyped 46,XX,t(2;16)(p13.3;p13.3). Her parents had normal karyotypes. Bazacliv et al(1973) reported the RTS patient with mosaic chromosome aberration included a deleted chromosome 2p in which breakpoints were not defined. These results suggest that the locus carrying the gene for the RTS may be at 2p13.3 rather than 16p13.3.

C 85

PATHOGENESIS OF CONGENITAL HEART DISEASE IN A CASE WITH MOSAIC TRISOMY 21. Yuji YOKOYAMA, Kouji NARAHARA, Kazushiro TSUJI, Hiroshi NAMBA, Tunenori MATSUBARA, Kazuhiro MORI, Yoshiki SEINO (Dept. Pediatr. Okayama University Medical School, Okayama)

To clarify the pathogenetic mechanism of congenital heart disease (CHD) in a patient with mosaic trisomy 21, we analyzed chromosomes from various tissues. This female patient was born at 38 weeks. She had many features of Down syndrome, and was diagnosed as having a karyotype of 46,XX/47,XX,+21(16%). At the age of 2 months, cardiac murmurs were noticed. UCG and cardiac catheterization revealed VSD+PDA+PH. Chromosome analysis on samples obtained by open heart surgery at the age of 5 years 4 months demonstrated the ratio of trisomic cells: 3.8% in peripheral blood lymphocytes, 33.3% in skin, 72.7% in lung and 90.5% in myocardium. GTG-banding analysis showed that the morphology of the short arm differed in each chromosome 21 of trisomic cells. QFQ-banding analysis of the patient and the parents revealed that trisomy 21 had resulted from maternal nondisjunction during meiosis I. We conclude that the mosaicism in this patient is originated from a fertilization ovum with trisomy 21. The occurrence of CHD seems to be related to the predominance of trisomic cells in the heart. The present study suggests that the tissue specific mosaicism of chromosome abnormality may be one of important pathogenetic factors of CHD.

C 86

ASSOCIATION OF DEL (11) (P13P11.2), ANIRIDIA, CATALASE DEFICIENCY, AND CARDIOMYOPATHY. Yoshifumi YAMAMOTO^{1,2}, Kou ICHIHASHI², Masayoshi YANAGISAWA², and Shigenori IKEMOTO³
(¹Dept. Pediatr. Tokyo Metrop. Police Hosp., Tokyo, ²Dept. Pediatr., ³Dept. Leg. Med., Jichi Medical School, Tochigi)

We report a second patient with del (11p), aniridia, catalase deficiency, and hypertrophic cardiomyopathy (HCM). Precise G-banding analysis, normal LDH isoenzyme pattern and only single copies of DNA detected by three c-DNA probes (D11S90, D11S93, D11S94) suggested that the deleted segment should be 11p11.2 to 13.

Gilgenkranz et al. (1982) reported the first case with association of del 11p, aniridia, catalase deficiency and HCM. Based on the normal electrophoretic LDH isoenzyme pattern, they concluded that the deleted segment was 11 p12~15.1. Recently, the LDHA gene is assigned to 11p14.3~15.1, so their case might have a deletion around the segment of 11p12. The etiology of HCM seem to be heterogeneous, but some of them are associated with other autosomal dominant condition. In addition, HCM may be clinically cryptic. Further cases need to be examined precisely by echocardiography to clarify the meaning of association of HCM and proximal 11p deletion.

C 87

CHROMOSOME CONSTITUTIONS IN 137 SPONTANEOUSLY ABORTED FETUSES AT A HIGHLY SUCCESSFUL CHROMOSOME ANALYSIS. Kodo SATO and Masumi OGAWA Department of Obstetrics and Gynecology and Department of Hematology, Toranomon Hospital, Tokyo.

Chromosome constitutions in spontaneously aborted fetuses were discussed at a highly success chromosome analysis rate. Chorionic villi from 138 spontaneous abortions were subjected to tissue culture for chromosome analysis, and satisfactory chromosome preparations were made in 137 (99.3%) fetuses. Sixty five percents of the fetuses were chromosomally abnormal; 60% of these were trisomic, 11% X-monosomics and 15% polyploid. Among the 52 trisomic fetuses, 25% were 16-trisomy, 21% 15-trisomy, 15% 22-trisomy, 8% 4-trisomy, 6% 21-trisomy, and so on. As the maternal age was advanced, incidence of trisomic fetuses was increased whereas the incidence of 45,XO fetuses was decreased. A total of 74 cases were examined at least twice between 6-8 week of pregnancy under vaginal ultrasonography to detect fetal heart movement. Depending upon the type of chromosome anomalies, or the number of trisomic chromosome, the incidence of abortions with history of positive fetal heart movement was changed. Many of X-monosomics, 15-, 21-, 22-trisomies were suggested to develop until the stage where fetal heart movement could be detected under vaginal ultrasonography.

C 88

CHROMOSOME STUDIES IN 30 COUPLES WITH SPONTANEOUS ABORTIONS Mikiko HAYASHI, Sachiko HONDA, Ichiro IYAMA, Shigeaki SATO (Toyama Institute of Health, Toyama), Yuchi NARUSE and Sadanobu KAGAMIMORI (Dept. of Community Medicine, Faculty of Medicine, Toyama Med. and Pharm. Univ., Toyama)

The frequency of chromosome anomalies in the couples with a history of recurrent abortions has been reported by a number of investigators to be 4.0 to 21.4%. In this work, chromosome studies were performed in 30 couples with a history of one or more spontaneous abortions before the 12th week of gestation. Five persons (16.7%) in different couples were found to have chromosome anomaly. Karyotypes of these cases were 45,XX,t(13;21), 46,XX,t(3;10)(q29;q22), 46,XY,inv(9)(p11;q13),t(4;10)(q31;q25), 46,XY,t(8;14)(p11;q32) and 46,XY,t(8;21)(q24.2;q21.2). In the case with a history of abortions, especially with recurrent abortions, chromosome studies in the couples seemed to be important in order to clarify the causes of abortion.

C 89

CYTOGENETIC STUDY OF SPONTANEOUS ABORTUSES AND ITS CLINICAL SIGNIFICANCE

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Yukari YANAGI², Masaaki ITO³, Hiroshi NAKAJIMA⁴ (¹Dept. Gyne. & Obst., Keio Univ.,
²Keio Health Cons.Center, ³Inagi City Hosp., ⁴Nakajima Clinic)

Cytogenetic study of 248 abortion specimens of 6-17 weeks gestation was performed by direct method, tissue culture method, or both. Chromosome preparations using trypsin-Giemsa banding technique could be obtained for 244 cases, 68% of which presented chromosomal abnormalities in number and/or in structure, among these, trisomy predominated (67%), followed by triploidy (13%) and monosomy X (9%). The prevalence rate of chromosomal abnormalities varied with the gestational age (77% until 12 weeks, 55% after 13 weeks), ultrasonographic findings (77% without fetus, 42% with fetus), past obstetrical history (79% in the first or second abortion, 57% in the third abortion or more) and maternal age (100% after 40 years old). In 77 cases with chromosomal abnormalities, 12% of the following pregnancy was also a miscarriage, whereas in cases without any abnormalities 34% ended in abortion. Repeated abortions in 16 cases are analyzed; of these, only 1 case showed the same chromosomal aberration.

C 90

CYTOGENETIC STUDY IN 11;19 TRANSLOCATED LEUKEMIAS

Miyuki SATO, Yasunobu YOKOYAMA, Reiko KAGAWA, Reiko ONISHI, Hiroko MATSUI, Yoshimori ISHIHARA, Kazumasa HIKIJI, and Yutaka TSUKADA (Genetic Research Lab. SRL., Inc. Hachioji)

We performed the cytogenetic investigations in 11;19 translocation associated leukemias. There were three types of 11;19 reciprocal translocation which involved the long arm of chromosome 11 and the short arm of chromosome 19- t(11;19)(q23;p13.1), t(11;19)(q23;p13.3), and t(11;19)(q13;p13.3), respectively. The first type were observed in M4 (four cases), M5a(one case), M5b(one case), M2(one case), RAEB in T(two cases), and as an additional chromosome aberration of CML in CP(one case). The median age of this type was 43.7 years old and it was higher than the age which have been mentioned that 11;19 translocation are especially more common in infant, previously. Moreover, our findings provide a hypothesis that t(11;19)(q23;p13.1) probably arises in a myeloid totipotent stem cell, which could differentiate into the granulocytic and monocytic pathways against the other two types might be in the multipotent stem cell. The second type were observed in one case of M5a and congenital acute leukemia(pre-B cell type. Even though it is quite few cases, it seems that the morphological feature is more variable in leukemic cells with this type of translocation than in those with other types which have been reported by Kaneko et al. Final type were observed in a case of AML(M2) with trilineage myelodysplasia and as an additional chromosome aberration of CML in CP.

C 91

CYTOGENETIC STUDIES OF 117 PATIENTS WITH ACUTE NONLYMPHO-CYTIC LEUKEMIA (ANLL). Kimie MIZUI¹, Michiko OKADA¹, Hideaki MIZOGUCHI² (¹Chromosome Lab., Shiseikai Dai-Ni Hosp., ²Div. Hematol., Tokyo Women's Med. Coll. Tokyo)

Chromosomes were studied on 117 patients with de novo acute nonlymphocytic leukemias (ANLL). Clonal chromosome abnormalities were present in 6 of 25 M1, in 29 of 44 M2, in 17 of 18 M3, in 11 of 17 M4, in 3 of 7 M5 and in 4 of 6 M6 patients. Present cases confirmed the already known association of t(8;21) with M2, t(15;17) with M3, 11q abnormalities with M4 and M5, and inv(16) with M4Eo. del(9q) was found in 1 M1 and 2 M2 cases. t(7;11) was also found in one M1 case. M2 patients involved 2 cases of t(16;21), one t(1;7) and one t(6;9) case. Specific association of these abnormalities with clinical and hematologic features was not evident in comparison of findings in our cases with those in the reported ones. In M3, M4, M5 and M6, patients with NN and AN chromosome pattern had longer survival time and better remission rate than those with AA pattern. Whereas, such correlation was not clear in M1 and M2. Relatively bad banding quality and low mitotic indexes in leukemic cells may have obscured chromosome aberrations. t(8;21) patients showed favorable prognosis both in AN and AA.

C 92

Ph POSITIVE LEUKEMIA, BEING IN CONFLICT WITH THE MANIFESTATION OF bcr REARRANGEMENT BAND, WHICH HAD i(9q+) IN LYMPHOBLASTIC CRISIS. Tamiko SHINOHARA¹, Yasuyuki INOUE², Kenji SUZUKI², Toshikazu YAMAGUCHI³ (¹Dept. Hum. Cytogenet., ²Dept. Internal Med., Japan Red Cross Med. Centr., Tokyo, ³Biomedical Lab., Saitama)

The cytogenetic analysis on bone marrow cells from a 30-year-old man with Ph positive leukemia, in which the bcr rearrangement had been detected with more faint bands than that of the germ line extremely, is reported. In May 1987 he was diagnosed as CML morphologically and cytogenetically, since Ph chromosome was in 100% of observed cells in bone marrow. When he relapsed in Nov. 1988, bone marrow aspirate contained 98.7% lymphoblasts, of which surface marker study showed that 90.8%, 90.3%, 45.8% and 96.4% of cells stained positively for CD10, CD19, CD20, and HLA-DR, respectively. Then chromosome analysis showed 47,XY,+8,t(9;22)(22%)/47,XY,+8,-9,+i(9q+),22q-(78%). He was treated with vincristine and prednisolone, resulting in a complete remission. Then He had normal karyotype(100%) in bone marrow cells. In Jan. 1990, he again relapsed. His WBC increased to 127000/mm³ with 98.5% lymphoblasts. The bone marrow aspirate was hypercellular with 98.6% lymphoblasts. The chromosome analysis showed 46,XY,t(9;22)(18%)/47,XY,+8,-9,+i(9q+),22q-(82%). Then the bcr rearrangement on bone marrow and on peripheral blood had been detected, but the abnormal bands were more faint in comparison with that of germ line. Even if he was in ALL, the disease was atypical.

C 93

CYTOGENETIC STUDIES IN THYROID TUMORS · Fumiko SAITO¹, Atsuhiko SAKAMO
TO², Kazuyoshi KAWABATA³, Nobukatsu KASAI³, & Akira TONOMURA¹ (1:Dept.
Genet., Inst. Med. Res., Tokyo Med. Dent. Univ., Tokyo, 2:Dept. Pathol., Tokyo
Univ., Tokyo, 3:Dept. Head & Neck, Cancer Inst., Tokyo)

Cytogenetic studies in thyroid tumors were performed by Q-banding of chromosome preparations obtained from the primary cultures of one adenoma and two papillary carcinomas. Various but non clonal structural aberrations were observed in one adenoma, but no chromosomal aberrations including #10 on which MEN2A gene is located were found. Trisomy 5, 7 and 8 were observed in carcinomas. These aberrations are of interest considering the recent observation that malignancy in thyroid tumor was associated with overexpression of the erbB1 oncogene mapped to #7 and alterations of the c-myc located in #8. The biological significance of various chromosomal changes in thyroid tumors remains to be elucidated by further studies of additional materials.

C 94

NEUROFIBROMATOSIS AND CHILDHOOD MALIGNANCY. Ichiro MATSUI, Masako
TANIMURA and Noboru KOBAYASHI (Natl. Children's Hospital, Japan Chil-
dren's Cancer Registry Center)

Neurofibromatosis (NF) is characterized by hereditary autosomal dominant disorders with high risk of malignancy in adult at the rate of nearly 14 percent. However, there has been little information on the risk of malignancy in childhood.

Among 21,097 cases stored in the database in Japan Children's Cancer Registry during 1969 and 1985, 30 cases of childhood malignancy with NF were found. Various types of malignancy distributed on various sites of the body from age 0 to 14 years, indicating a risk of malignancy even in childhood.

We compared the cancer type in NF children with that in 531 adult autopsied cases of the data from the Annual of the Pathological Autopsy Cases in Japan. Epithelial cancers frequently observed in NF adults were not found in NF children. However, leukemia (9 cases), rhabdomyosarcoma (4 cases) or the like, peculiar to childhood, were rarely observed in NF adults. Brain tumors and peripheral nerve tumors were encountered in NF children as well as NF adults. NF gene may play a role in oncogenesis in different organs at each stage of life.

C 95

A STUDY ON FAMILY HISTORY OF CHILDREN'S CANCER. Masako TANIMURA, Ichiro MATSUI and Noboru KOBAYASHI (Nat'l. Children's Hospital, Japan Children's Cancer Registry Center)

Childhood malignancy is considered to be more closely related to congenital factors involved in genetic or intrauterine environmental background than to postnatal environmental factors. The association with genetic factors, however, has been reported in only a few childhood malignancies because of early onset and the rare familial case.

We found some patients whose sib or cousin had the same type of malignancy as proband, among 12,016 cases diagnosed between 1979 to 1987 in the Japan Children's Cancer Registry database. These were 1) retinoblastoma, neuroblastoma and Wilms' tumor, in which loss of heterozygosity has been already found, 2) familial erythrophagocytic histiocytosis known as an autosomal recessive, and 3) ocular rhabdomyosarcoma, histiocytosis X, AMoL, Hodgkin's disease and insulinoma, which were newly suggested to be related to genetic factors. Consanguinity and case reports suggested a possible inheritance mode for rhabdomyosarcoma, histiocytosis X as dominant and for Hodgkin's disease as recessive.

C 96

NONRANDOMNESS OF SPONTANEOUS CHROMOSOME BREAKPOINTS IS RELATED TO MALIGNANT NEOPLASMS IN FANCONI ANEMIA Kazushiro TSUJI, Kouji NARAHARA, Yuji YOKOYAMA, Megumi ODA, Ayumi AKAZAI, Ritsuo NISHIUCHI, Yoshiaki SEINO Dept. Pediatr. Okayama University Medical School

We analyzed response to mitomycin C (MMC) and spontaneous chromosome breakpoints in lymphoblastoid cells or skin fibroblasts established from 4 patients with Fanconi anemia (FA), to solve the question why FA is associated with a particular type of neoplasm. Two patients with acute myelocytic leukemia (AML) showed nonsensitivity to MMC, whereas the 2 other patients with multiple congenital anomalies had hypersensitivity to MMC. Analysis of spontaneous breakpoints showed that the frequency of breakage was 4 to 5 times higher in the FA patients than in normal controls. The breakpoints tended to gather on the heterochromatic and paracentromeric chromosome regions. Significantly more breakpoints had occurred on the 1p11 or q11, 1q21, 3p14, 3q21, 7p11 or q11, 8q22, 9q11-q22, 11q23 and 12q13 bands. The coincidence between the spontaneous breakpoints in FA and those in patients with acute nonlymphocytic leukemias suggests that the nonrandomness of breakpoints may play an important role in the tumorigenesis of FA. Future molecular study on oncogenes neighboring the specific breakpoints in malignant cells from FA patients is required.

C 97

SUPPRESSION OF TUMOR CELL PROLIFERATION BY INTRODUCTION OF A NORMAL CHROMOSOME #3p INTO HUMAN RENAL CELL CARCINOMA CELL LINE. Mitsuaki A. YOSHIDA¹, Tatsuro IKEUCHI¹, Akira TONOMURA¹, Motoyuki SHIMIZU², Hiroyuki KUGOH², Mitsuo OSHIMURA² (¹Dept. Genet., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo, ²Dept. Mol. & Cell Genet., Sch. Life Sci., Tottri Univ., Tottri)

For better understanding the role of #3p in the development of renal cell carcinoma (RCC), we introduced a single human chromosome containing #3p into a human RCC cell line, "RCC23", established in our laboratory. The donor chromosome consisted of 3pter-3q13 and unknown segments. The recipient RCC23 cells showed an unbalanced translocation 3p;8q, leading to a loss of the 3p11-pter region. A total of 9 microcell hybrid clones were obtained, and 5 of them were examined for their phenotypic properties. Three clones with an intact transferred #3p showed a marked morphological change and a significant decrease in the growth rates in both usual cultures and soft agar plates. One clone showed a partial loss of the transferred #3p, and revealed a decreased growth rate, but no change in morphological appearance. On the contrary, there were no phenotypic alterations in a hybrid clone containing an extra small chromosome probably derived from the transferred #3p, and in clones with a normal chromosome #7 transferred as controls. Our findings indicate that a tumor suppressor gene(s) possibly associated with the human RCC development may exist within a limited region of the chromosome #3p.

C 98

ULTRASOUND ESTIMATION OF HUMAN KIDNEY GROWTH FROM FETAL PERIOD TO ADULTHOOD. MATSUI, A.*
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Ultrasound estimation of human kidney growth was performed in 411 normal subjects from 17 weeks' gestation to 30 years of postnatal age. The anteroposterior mean diameters of bilateral kidneys were 1.2 cm at 17-20 weeks' gestation, 2.4 cm at 39-40 weeks' gestation, 2.2 cm at neonatal period, 4.0 cm at 10-12 years, and 4.7 cm at 25-30 years. The respective longitudinal kidney mean diameters were 2.0 cm, 4.3 cm, 4.7 cm, 9.2 cm, and 10.7 cm. These two diameters were highly correlated with age, body weight, body height, head and abdominal circumference.

Ultrasound scanning is useful for assessment of human kidney growth as well as early detection and diagnosis of kidney and urinary tract abnormalities.

C 99

ANOPHTHALMOS ASSOCIATED WITH SPHENOETHMOIDAL MENINGOENCEPHALOCELE, AGENESIS OF CORPUS CALLOSUM, CLEFT LIP AND PALATE. Akihiro KURIMASA/1, Masumi INAGAKI/1, Kyoichi OHTANI/2, Kousaku OHNO/1, Kenzo TAKESITA/1 (Div. Child Neuro., Inst. Neuro. Sci., Tottori Univ., Sch. Med., Yonago/1, Tottori Pref. Cent. Hosp. Tottori/2)

An association of sphenothmoidal encephalocele, agenesis of the corpus callosum (ACC) and midline facial anomalies (Sakoda complex: SC) has been proposed as a distinct type of congenital anomaly (Sakoda et. al., 1979). Tada et. al. (1985) described a case with the Sakoda complex, anophthalmos and neonatal seizures, suggesting a frequent association of ocular anomalies in this complex. We have identified the similar case as described by Tada et al..

A male patient was born to unrelated healthy parents at 36 weeks gestation, weighting 2850 gm. Cleft lip and palate, and bilateral anophthalmos were noticed at birth and seizure appeared within 24 hours. At 15-month-old he showed severe psychomotor delay and seizures on right side. CT and MRI showed ACC, sphenothmoidal bone defect with encephalocele, atrophy of the left hemisphere and rudimentary eye balls. Chromosome was a normal male karyotype.

We have noticed that our case showed marked similarity with case described by Tada morphologically and clinically, and distincted from SC with other ocular dysplasias. It is suggested that SC with bilateral anophthalmos is a distinct clinical entity.

C 100

Worldwide Records Gathering at The Genealogical Society of Utah

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The Genealogical Society of Utah. 50 East North Temple Street, Salt Lake City, Utah 84150 U.S.A.

Collection of The Genealogical Society of Utah includes nearly 1.5 million rolls of microfilmed records, 200,000 microfiche, over 195,000 books, 8 million Family Group Record forms, and many other records. These records are available the main Library and hundreds of Family History Centers all over the world. The records have been acquired through an extensive microfilming program that began in 1938. They are filming in courthouses, churches, and other archives in forty-five countries. The International Genealogical Index is a computerized index of named extracted from various records. The 1988 edition lists the names of over 121,000,000 deceased persons. It is available on microfiche or CD-ROM.

C 101

SEX DIFFERENCE IN CARRIERS OF INV(9) CHROMOSOMES. Kiyomi YAMADA (Div. GENETICS, Clin Res Inst., Natl Med Center Hosp., Tokyo)

Using C-bandings by a DA-DAPI double stain method, the population incidence of individuals carrying a pericentric inversion of No. 9 chromosome was studied in a total of 3,702 individuals from the following 6 populations; normal healthy volunteers (NR; n=1,513), Down syndrome (DW; n=1,190), Klinefelter syndrome (KF; n=251), azoospermia (AZ; n=230), couples with repeated abortion history (HA; n=210), and spontaneously aborted fetuses (SA; n=308).

Apparent sex difference in carriers of inv(9) chromosomes was revealed by the present study: Female incidences of 0.021 in NR and 0.020 in DW were significantly higher than male incidences of 0.012 in NR and 0.011 in DW. Interesting was the fact that KF male incidence of 0.024 was very close to an incidence of normal females, while AZ male incidence of 0.013 was in the normal male range.

Population incidences of 0.033 in HA and 0.030 in SA with normal karyotype were relatively higher, when compared with NR incidence of 0.017.

Sex difference and biological meanings of individuals carrying inv(9) chromosomes were discussed.