日本人類遺伝学会 第34回大会一般講演要旨

Abstracts of General Contribution, the 34th Annual Meeting of the Japan Society of Human Genetics

A 1

STUDIES ON AN ACCESSORY TRANSCRIPTION FACTOR, FE, USED BY RNA POLYMERASE II. Tatsuo KAWAGUCHI, Shigetaka KITAJIMA, Yukio YASUKOCHI and Sherman M. WEISSMAN* (Dept. Human Genet., Tokyo Med. Dent. Univ., Tokyo; *Dept. Human Genet., Yale Univ., CT, USA)

A transcription factor (FE) required for specific transcription by mammalian RNA polymerase II was purified from HeLa cell nuclear extract after successive column chromatographies, including DEAE-Sepharose, phosphocellulose, DEAE-Toyopearl 650, Sephacryl S-300, DEAE-HPLC, and TSK250 HPLC gel filtration columns. The last one, which was performed in the presence of 4 M guanidine-hydrochloride followed by renaturation, could identify that the FE activity resides in fraction composed of a 33 KDa polypeptide as a major constituent. The activity was inactivated by heat with some resistance and sensitive to treatment with 10 mM N-ethylmaleimide. Sarkosyl block assay indicated involvement of FE in a rapid phase of reaction after formation of initial complex. Furthermore FE was shown to specifically associate with purified calf thymus RNA polymerase II.

A 2

PURIFICATION OF DNA BINDING PROTEIN IN HeLa CELL NUCLEAR EXTRACT. Takayuki NAGAOKA, Shigetaka KITAJIMA*, Ikuhisa SAWADA* and Yukio YASUKOCHI* (Dept. Clin. Genet., Kyorin Univ. Sch. Health Sci., Tokyo; *Dept. Human Genet., Tokyo Med. Dent. Univ., Tokyo)

During our efforts to isolate the accessory transcription factor which interacts with the TATA motif, we found a DNA binding protein in HeLa cell nuclear extract. The DNA binding protein was co-purified with the transcription activity of FC till Sephacryl S-300 HR gel filtration as measured by gel-mobility-shift assay. However, further chromatography on a TATA oligonucleotide column separated the FC preparation into the DNA binding activity which interacted with the column tightly but was distinct from the transcription activity of FC. The DNA binding activity resided in a 120 KD peptide when protein blotting was performed using radiolabeled β-globin promoter sequence. The amino acid sequence of a tryptic peptide was determined to be Phe-Asn-Pro-Leu-Asn-Thr-Asn-Gln-Phe-Tyr-Ala-Ser. To investigate possible function of this protein, gene cloning is under way.

A 3

LOCALIZATION OF THE HUMAN CHROMOSOME 6 Not I LINKING DNA. I.SAWADA[1], A.TAM[2], H.ZOGHBI[3], C.M.WITKOWSKI[4], J.M.TRENT[4], Y.YASUKOCHI[1] and S.M. WEISSMAN[5] (1.Tokyo Med. & Dent. Univ., Tokyo; 2.Genelabs, CA; 3.Baylor College of Med., TX; 4.Univ. of Arizona, AZ; 5.Yale Univ., CT, USA)

We have developed a new method for the preparation of chromosome specific Not I linking libraries. Available human chromosome 6 specific lambda libraries were first transfered to a plasmid vector and supercoiled library DNA was prepared. After digestion with Not I, the DNA was ligated with kanamycin gene DNA fragment. Only clones linealized by Not I digestion were recovered after transformation and selection by kanamycin. Prepared extensive sets of Not I linking DNA were then localized by hybridization using human/rodent hybrid panels. At least 10 different clones were localized on the short arm and 29 clones on the long arm of the human chromosome 6. These clones show no extensive polymorphism (except one) and many of them cross-hybridized with rodent genomic DNA. This observation suggests that evolutionarily well preserved regions can be identified by the clones. We are now investigating the genome organization surrounding the human MHC and myb oncogene loci using Not I linking DNA.

A 4

CLONING AND CHARACTERIZATION OF AN X-Y HOMOLOGOUS AMELOGENIN GENE. Yutaka NAKAHORI[1], Takashi TAMURA[1,2], Koichi HAMANO[1], Masao YAMADA[1], Yasuo NAKAGOME[1], Osamu TAKENAKA[3] ([1]Dept.Cong.Abn.Res., Nat.Child.Med. Res.Ctr.; [2]Dept.Med.Genet., Kyorin Univ. School Hlth.Sci.; [3]Primate Res.Isnt., Kyoto Univ.)

An Xp-Yq homologous DNA fragment (87-4) cloned from a flow sorted human Y-specific lambda library has been found to be highly conserved during the evolution of mammals. It was conserved on both the X and Y chromosomes in some species of old-world monkeys, a new-world monkey and bovine, and only on the X in most other monkeys, mice and rats. The X-chromosome homologue was also cloned and the most conserved parts were sequenced. Both the X- and the Y- chromosome sequences were found to have open reading frames which showed homology to the amelogenin gene reported in mouse and bovine. Both had 3 exons. Though it is hard to tell whether the Y sequence is active or not, the fact that exons are more conserved than the surrounding sequences suggests that the Y sequence is either working or had been working until very recently in terms of evolution.

A 5

TOWARD PHYSICAL MAPPING OF HUMAN Y CHROMOSOME. Takashi TAMURA[1,2], Yutaka NAKAHORI[1], Masao YAMADA[1], Yasuo NAKAGOME[1], Toshiyuki FURUSHO[2], Yoshimitsu FUKUSHIMA[3], Shigeru MINOWADA[4], Kenji FUJIEDA[5], Yoshikazu KUROKI[6] ([1]Dept.Cong.Abn.Res., Nat.Child.Med.Res.Ctr., Tokyo; [2]Dept. Clin.Genet., Kyorin Univ. School Hlth.Sci., Tokyo; [3]Saitama Child. Med.Ctr., Iwatsuki; [4]Dept.Urol., Univ.Tokyo; [5]Dept.Pediat. Univ. Hokkaido, Sapporo; [6]Kanagawa Child.Med.Ctr., Yokohama)

Ten novel DNA probes detecting 11 different sites on the human Y chromosome were physically mapped within the non-fluorescent region of the Y(p11-q11). Using 4 previously known probes in addition to 7 of our novel probes, the interval 5-6 can now be divided into 10 subregions. An attempt to establish possible correlation between the presence or absence of a particular subregion and that of a clinical symptom, such as azoospermia or short stature, is in progress.

A 6

A SIMPLE MICRODISSECTION/MICROCLONING METHOD FOR CLONING OF CHROMOSOME REGION-SPECIFIC DNA. (FIRST OF TWO PARTS): MICRODISSECTION. Naoki HARADA, DENG Han-Xiang, Tetsuya HIROTA, Jun-ichi HAMABE, Kazuhiro TSUKAMOTO, Yoshihiro JINNO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

A method of chromosome microdissection for cloning of chromosome band-specific DNA is presented. Prometaphase chromosome (550 band-stage) made with synchronization culture method with only thymidine was dissected under an inverted microscope with a micro glass needle (0.3um tip) attached to three-dimensional micromanipulator(Narishige). Five pieces of chromosome fragments dissected for an identical band were collected in a 100 nl drop of proteinase K solution in an oil chamber. After collection, the solution was transferred into a micro-centrifuge tube. Further DNA manipulations described in the next paper were all performed within the tube. Five chromosome pieces could be cut out and collected within half an hour and a total of 1-5 x 10^5 clones with the average size of 250bp were obtained within 3 days. This microdissection method allows to avoid most small-scale steps of work described in previous studies by other investigators and to use routinely made chromosome slides without any cares of cell fixation condition. It is applicable to not only the cloning of chromosome region-specific DNAs but also regional gene mapping.

A 7

A SIMPLE MICRODISSECTION/MICROCLONING METHOD FOR CLONING OF CHROMOSOME REGION-SPECIFIC DNA (SECOND OF TWO PARTS): MICROCLONING. Tetsuya HIROTA, Kazuhiro TSUKAMOTO, Yoshihiro JINNO, Jun-ichi HAMABE, Naoki HARADA, DENG Han-Xiang, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

A method of simple and rapid cloning of DNAs from dissected chromosomes is reported: (1)Dissected chromosome fragments were collected and digested in 200 nl of proteinase K solution, (2)the solution was transferred into an Eppendorf tube, (3) DNA was cut by NlaIII and (4)ligated to a 20mer oligonucleotide with (5')CATG(3') at its 3'end and an EcoRI recognition site near 5'end, (4)ligation product underwent PCR using the same oligonucleotide in excess as a primer, and (5)PCR product was ligated to EcoRI-cut pUC19. A total of 2.1 x 10^5 clones were obtained from 5 pieces of chromosome region, 8q23-24.1, where genes responsible for tricho-rhino-phalangeal syndrome type I (TRPSI) and TRPSII may exist. Among 36 clones analyzed, the mean size of insert was 250 bp. Of 11 clones screened with the slot-blot method to hybridize to genomic DNAs from both TRPSI and II patients, 1 showed 1-copy density for both patients and another showed 1-copy density for only TRPSII patient, suggesting a candidate clone for the exostosis gene. This method has the following advantages:the first ligation reaction is independent on the concentration of chromosomal DNA, all steps can be performed within an Eppendorf tube, and a cloning of band-specific DNAs can be achieved within 3 days.

A 8

PURIFICATION OF M-BsuE AND ITS USAGE FOR MODIFICATION OF NotI RESTRICTION SITE. Y. TANAKA, H. ARENSTORF*, S. KITAJIMA, S.M. WEISSMAN*, and Y. YASUKOCHI (Dept. Human Genet., Tokyo Med. Dent. Univ., Tokyo; *Dept. Human Genet., Yale Univ., New Haven)

The endonuclease NotI has been used to generate large fragments of DNA for the physical mapping of mammalian genome. The restriction modification enzyme M-BsuE can be used to increase the NotI fragment size by converting the restriction site to GC/GGCC^{m}GC. The M-BsuE has been isolated from Bacillus subtilis strain ISE15 by streptomycin precipitation and column chromatography on DEAE-Sepharose, DEAE-HPLC, and Heparin-HPLC, which achieved a 1300-fold purification. DNA methyltransferase activity was monitored radiochemically and was confirmed by BstUI protection. An SDS-PAGE showed a major 44 KDa peptide, from the N-terminal of which amino acids have been sequenced. The NotI protection experiment, using a plasmid construct, pGEM + Not + KanR, showed that the NotI sites were completely blocked even it only over-laps a M-BsuE site at one end instead of at both ends of the NotI site.

A 9

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) AND RIBONUCLEASE CLEAVAGE METHOD (RNase METHOD) FOR DETECTING VARIATIONS IN DNA.

Chiyoko SATOH, Norio TAKAHASHI, Keiko HIYAMA, Mieko KODAIRA (Dept. Genet., Radiation Effects Research Foundation, Hiroshima)

We have examined the feasibility of DGGE and RNase method for a study to detect variations in DNA. In the first step, identical RNA:DNA duplexes (dpx) of approximately 500 base pairs (bp) made from RNA probes and fragments from human β-globin genes in cloned or PCR-amplified genomic DNA samples were examined with the 2 methods. They detected the same nucleotide substitutions, deletions or insertions.

A fragment of 870 bp which includes the sequence between positions 23 in exon 3 and 2237 from the capping site of the human β-globin gene was amplified by PCR. A HaeIII fragment (476 bp) of the product was directly examined by the DGGE. Two new polymorphic substitutions of G to A and C to A at positions 1787 and 1789, respectively, were detected. The DGGE of RNA:DNA dpx made from the HaeIII fragment also detected them. The RNase method detected a new polymorphic A to T substitution at position 1945 and the 2 substitutions without recognizing difference between them. Since the efficiency of the 2 types of the DGGE seems to be equal, the DGGE on PCR-amplified DNA fragments is the most suitable among the 3 methods for screening for DNA variations since neither probes nor radioisotopes are necessary.

A 10

USE OF TRANSGENIC MICE FOR DISSECTING THE MOLECULAR MECHANISM OF AMYLOID DEPOSITION IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY.

Takeaki INOMOTO*, Fumi TASHIRO*, Shoji WAKASUGI*, Ken-ichi YAMAMURA*, Shigehiro YI**, Takifumi MURAKAMI, Shuichiro MAEDA, Kazunori SHIMADA (Inst. for Med. Genet.*, Dept. of Pathol.**, Dept. of Biochem., Kumamoto Univ. Med. School, Kumamoto)

It is clear that the main cause of familial amyloidotic polyneuropathy(FAP) is the presence of mutant transthyretin gene(TTR) which is resulted in the production of variant TTR molecule leading to the systemic amyloid deposition. To analyze the pathological process of amyloid deposition we have produced two lines of transgenic mice, carrying either human mutant TTR gene with its own promoter (0.6-hTTR30) or with metallothionein promoter(MT-hTTR30).

Amyloid deposition was observed in many tissues including alimentary tract, kidney, heart, skin and some other tissues.

These results suggest that the transgenic approach is useful for establishing the mouse model for FAP, and that these transgenic mice, could be used for dissecting the mechanism of amyloid deposition in FAP.

A 11

EXPRESSION OF HUMAN MUTANT TRANSTHYRETIN GENE IN TRANSGENIC MICE.
Fumi TASHIRO[1], Takeaki INOMOTO[1], Shigehiro YI[2], Tatsufumi MURAKAMI[3], Shuichiro MAEDA[3], Kazunori SHIMADA[3], and Ken-ichi YAMAMURA[1] ([1]Inst. Med. Genet., [2]Dept. Pathol., [3]Dept. Biochem., Kumamoto Univ., Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder characterized by extracelllular deposition of amyloid fibrils and by peripheral and autonomic nerve involvement. To analyze the mechanisms of amyloid deposition, we have produced two lines of transgenic mice by microinjecting human mutant transthyretin gene, with either its own promoter (0.6-hTTR30) or metallothionein promoter (MT-hTTR30). Amyloid deposition was observed in various tissues of both lines, but not in peripheral nerve. Then, we produced another line of transgenic mice in order to increase the level of transgene expression (6.0-hTTR30). The serum level of human variant TTR in 6.0-hTTR30 mice ranged up to 17 mg/dl, and was higher than in human FAP patient. The 6.0-hTTR30 gene was expressed in liver, brain and kidney similarly as the endogenous gene. Developmental analysis showed that mRNA of transgene was detected at 13 days of gestation. Compared to 0.6-hTTR30 mice, the level of transgene expression in liver was 8-fold and expression in choroid plexus was observed by RNA in situ hybridization. Amyloid deposition in 6.0-hTTR30 mice began at 9 months of age that was 6 months earlier than that of 0.6-hTTR30 mice.

A 12

MOLECULAR BASIS OF β-THALASSEMIA IN THAILAND.
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β-Thalassemia genes in 71 chromosomes of the Thai patients who inhabited in three different regions of Thailand, the Northeast, the Middle and the South, have been investigated by dot blot hybridization of the PCR-amplified DNA with allele-specific oligonucleotide probes. Eight different known molecular defects were detected at different frequency. They are an amber mutation in codon 17, a C-T transversion at position 654 of IVS-2, a frameshift mutation between codons 71-72, an A-G transition at nucleotide -28 within the TATA box known as Chinese mutations, a G-T transversion at position 1 of IVS-1 known as an Indian mutation, a 4 bp deletion in codons 41-42 and a G-C transversion at position 5 of IVS-1, which were descibed as both Chinese and Indian mutations and a Thai original mutation, an ochre mutation in codon 35. Analysis of the three unknown alleles by DNA sequencing of the cloned DNA fragment amplified by PCR revealed an A-G substitution at the second position of the codon for an amino acid 19 (AAC-AGA). As this novel mutation occurs within the cryptic donor splice site, the mutation would therefore affect β mRNA metabolism by activating the use of this cryptic site and a β^+-thalassemia phenotype would follow. Only Chinese mutations were observed in the Northeast, while both Chinese and Indian mutations were detected in the Middle and the South of Thailand. Two kinds of Thai mutations were found in the Middle and the South, respectively. The approach used in this paper and the characteristic distribution of mutations in each region of Thailand are useful for the planning of prenatal diagnosis program in Thailand.

A 13

DIAGNOSIS OF APRT DEFICIENCY USING DNA AND ESTIMATION OF THE TIME OF *APRT*J* MUTATION. Naoyuki KAMATANI, Shoko KUROSHIMA and Masayuki HAKODA. Institute of Rheumatology, Tokyo Women's Medical College, 2-4-1 Nishishinjuku, Shinjuku-ku, Tokyo, Japan

Most of the reported cases with adenine phosphoribosyltransferase (APRT) deficiency causing 2,8-dihydroxyadenine (DHA) urolithiasis and renal insufficiency have been Japanese. In addition, approximately 80% of all the Japanese homozygous individuals have a single mutant allele designated *APRT*J* with a base substitution causing a change from ATG to ACG at codon 136 (Hidaka et al.). We have attempted to identify this mutation by the specific oligonucleotide hybridization after *in vitro* amplification of a part of human APRT genomic sequence (PCR method), and calculate the time of mutation by the analysis of dissolution of the linkage disequilibrium. Results of the specific oligonucleotide hybridization were the same as our previous data about the genotypes of individuals obtained by the T-cell diagnostic method and the enzyme assay with hemolysates. The haplotype analysis for each *APRT*J* allele has shown that the specific mutation sequence showed 100% association with a TaqI- RFLP for a site located about 1.1 kb upstream of the mutation site, and 84% association with a SphI+ RFLP for a site located about 3.8 kb upstream of the mutation site. Normal APRT alleles were distributed roughly evenly into 4 haplotypes. If the rate of crossovers between the *APRT*J* mutation site and the SphI RFLP site has been average, the origin of the *APRT*J* mutation is calculated to be approximately 160,000 years ago under certain assumptions.

A 14

DNA DIAGNOSIS IN JAPANESE FAMILIES WITH MYOTONIC DYSTROPHY USING POLYMORPHIC DNA MARKERS

Tetsuro MIKI, Yuji TAKEMOTO, Kumi NISHIKAWA, Jun NAKURA, Kouzin KAMINO, Toshio OGIHARA (Dept. Geriat. Med., Osaka Univ.) Shin-ichiro TAKAI (Second Dept. Surg. Osaka Univ., Osaka) Takeshi YAMADA (Dept. Neurl.,Kyusyu Univ.,Fukuoka) Masanori NAKAGAWA (Nat.Sanat. Okinawa Hosp:,Okinawa) Itsuro HIGUCHI and Mitsuhiro OSAME (Third Dept. Int.Med., Kagoshima Univ., Kagoshima)

Myotonic muscular dystrophy (DM) is a severe neuro-muscular disorder and inherited in an autosomal dominant fashion. The incidence of DM is approximately 5 per 10^5 in both Caucasian and Japanese populations. Neither the gene responsible for DM or its basal disorder has yet been identified. We have reported that in Japanese families the DM locus is also closely linked to D19S19 and APOC2 on chromosome 19. Since age of onset and the clinical symptoms and signs are different among not only the DM patients from unrelated DM families but also affected members of the same DM family, it is difficult to detect a presymptomatic patient or an asymptomatic carrier in DM families by conventional clinical examination. We have carried out the preclinical detection of a gene carrier in Japanese DM families by linkage analysis using APOC2 and D19S19 as the genetic markers .

A 15

CONSTRUCTION OF CHAROMID LIBRARIES DERIVED FROM A HYBRID CELL WHICH CONTAINS THE PORTION OF CHROMOSOME 19, 19q13.2.

Yuji Takemoto, Tetsuro Miki, Jun Nakura, Kouzin Kamino, Kumi Nishikawa, Toshio Ogihara, (Dept.Geriat.Med.,Osaka Univ., Osaka) and Shin-ichiro Takai (Second Dept. Surg.,Osaka Univ.,Osaka)

Myotonic muscular dystrophy (DM) gene is located on the long arm of chromosome 19, 19q13.2. For the purpose of cloning the DM gene, we have constructed charomid libraries from a hybrid cell between human cell and Chinese hamster ovary mutant deficient in DNA repair gene, 20xp0435-2, which contains the ERCC-1 gene and the DM gene. DNA from this hybrid cell were partially digested with HindIII and about 30 and 15kb DNA fragments were ligated to HindIII sites of charomid 9-20 and 9-28 respectively. Ligated DNA were packaged and infected to ED8767. We have got $3x10^5$ recombinant clones from these libraries and screened with total human DNA. In $5x10^4$ clones screened, we have got 54 clones by second screening and have been analyzing them by gene dosage effect.

A 16

NOTI LINKING LIBRARIES OF HUMAN CHROMOSOME 19.

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Yashufumi KANEDA (Inst. Mol. Cell. Biol., Osaka Univ., Osaka)
Sin-ichiro TAKAI (Second Dept. Surg., Osaka Univ., Osaka)

The gene responsible for myotonic muscular dystrophy (DM) is located to the long arm of chromosome 19 by linking analysis. There seemed to be about 50 NotI sites on chromosome 19 because chromosome 19 is one of the smallest chromosome. We have constructed NotI linking libraries derived from the human 19 phage library. In the three clones selected from this libraries, we have mapped two clones to the long arm and one to the short arm. These linking clones were used as probe in the following experiments. RFLPs for each clones was examined with DNA, digested by 9 different enzymes, from unrelated 6 Japanese individuals. pKNB46, which was mapped to 19q, has BglII RFLPs. In order to further analysis of these linking clones we have carried out linkage and physical mapping by using C.E.P.H. families and PFGE.

A 17

PHYSICAL MAPPING AROUND THE MYOTONIC DYSTROPHY LOCUS BY PULSED FIELD GEL ELECTROPHORESIS

Jun NAKURA, Tetsuro MIKI, Yuji TAKEMOTO, Kumi NISHIKAWA, Kouzin KAMINO, Toshio OGIHARA (Dept. Geriat. Med.,Osaka Unv., Osaka) and Shin-ichiro TAKAI (Second Dept. Surg., Osaka Univ., Osaka)

It has been reported that the gene responsible for myotonic muscular dystrophy (DM) is located to the long arm of chromosome 19 (19q13.2), between the region including CKMM (creatinine kinase, muscle form) gene and ERCC1 (DNA repair gene 1) and that including anonymous polymorphic DNA probes, D19S50 and D19S22 by linkage analysis. The distance of two regions would be too long to analyze by using a conventional gel electrophoresis. In order to develop an accurate physical map around DM locus, we have constructed a rare-cutter restriction enzyme map using pulsed field gel electrophoresis (PFGE), the LKB Pulsaphor hexagonal system. We have mapped the ERCC1 within 380 kb distal to the CKMM.

A 18

MOLECULAR CLONING AND DISSECTION OF GROUP A XP GENE.

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We isolated the gene responsible for xeroderma pigmentosum (XP) group A by DNA transfection method. Northern blotting of poly A^+ RNA from group A through G(H), variant XP and normal human cells using this gene(XPAC gene) as the probe revealed that 1.0kb and 1.4kb mRNAs were present in group B through G(H), variant XP and normal human cells, but absent in almost all group A XP cell strains. Instead, aberrant mRNAs were present in almost all group A XP cells. Southern blotting analysis revealed no gene rearrangements in XPAC genome of group A XP cells tested, indicating that mutation in group A XP cells might be a point mutation in the exons, exon-intron junctions, or regulating regions. Human XPAC cDNA encoded 645bp ORF and deduced M.W. of XPAC protein was about 25K daltons. No significant homologies were observed in a comparison of XPAC protein sequence with the protein sequences reported so far. Human XPAC gene was assingned on chromosome 9 q22 by in situ hybridization method(M. Yoshida, Hokkaido Univ.).

A 19

STRUCTURE AND ORIGIN OF A METACENTRIC MARKER CHROMOSOME IN TETRASOMY 18p SYNDROME.
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Tetrasomy 18p syndrome is a distinctive clinical syndrome with an extra metacentric marker chromosome, the presence of which causes various developmental abnormalities including psychomoter disturbances and muscular hypertonia as a sign of lesion of the pyramidal system. Southern hybridization with L2.7 probe, which is known to be located in region 18p indicated that an increase in the copy number of sequence in 18p region as compared to those of β-globin gene. In order to know further the difference between i(18p) and 18q- chromosomes with an equal size, we attempted to clone the telomeric repeat and the flank sequences by the use of polymerase chain reaction (PCR) technique, which resulted in obtaining a large number of clones. Since sequences unique to each of the chromosomes have to be identified yet, we tentatively conclude that the structure of the marker chromosome is just as has been predicted, i.e., i(18p).

A 20

ANALYSES OF BREAKPOINTS OF RECIPROCAL TRANSLOCATION CHROMOSOMES WITH BALANCED CONDITIONS IN MAN (2).
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The frequencies of autosomal rearrangements with balanced reciprocal translocation ascertained for only one of parents through probands in each family were estimated from our records and published data (OB). In 216 families examined, male and female probands were 116 and 99, respectively, while their fathers and mothers with the balanced conditions were 78 and 138, respectively. The data were compared with those in cases of recurrent abortion (RA) and in cases with balanced reciprocal translocation ascertained for two or more generations (BB).

The analyses of the cytogenetic finding suggest that some chromosomes are preferentially involved and that the breakpoints are not distributed at random on the chromosome arms. These tendencies in OB and BB are much higher than that in RA. In particular, there is an excess or dearth of breakpoints on the same 11 arms in both OB and BB, but only 2 arms in OB and RA. A characteristic excess or dearth of those in OB, BB and RA is also noticed on 2, 1 and 3 arms, respectively. From these data it is considered that the most cases of OB and BB are classified in the same category and these of RA in the other.

A 21

DNA REPLICATION PATTERNS IN A PSU DIC(X) CHROMOSOME ATTACHED BY THEIR LONG ARMS. Eiko ARAI, Akira TONOMURA (Dept.Genet., Med.Res.Inst., Tokyo Med.Dent.Univ., Tokyo), Mitsushiro KIDA (Dept.Pediatr., Teikyo Univ., Tokyo) and Takao OHTA (Dept.Obstet.Gynecol., Teikyo Univ., Tokyo)

The patient presented with primary amenorrhoea at the age of 20 years. Her intelligence is normal. On examination, her height was 149cm and weight was 39kg. Secondary sexual development was poor, webbed neck and cubitus valgus were negative. The uterus was infantile. Endocrine examination revealed that gonadotropin was a low level. Cytogenetic studies were proposed that her karyotype could be written as 45,X/46,X, psu dic(X:X)(pter→cen→q22::q22→pter). In literatures, about 20 cases of dic(X) with breakpoint on the long arm have only been reported. Studying the DNA replication pattern on both sides of the conjunction point of the X chromosome, somewhat asynchronous behavior was found in our case. Furthermore, two types of the asymmetric replication pattern were observed, that is, the replication started earlier and later on the side of the single and active centromere. So far as we know, the asymmetric replication patterns were reported in 3 cases, although the different mechanism were proposed respectively. In our case, the presence of different asymmetric replication patterns in the terminal segment of the short arms seems to be arisen by random inactivation in one of the two centromeres during early outogeny.

A 22

PRENATAL DIAGNOSIS OF POTTER'S TYPE I CYSTIC KIDNEY DISEASE IN 2 SIBLINGS. Akira Matsui (Dept. Pediat.,Isesaki Municipal Hosp., Gunma), Tsunehisa Takenaka (Dept. Obstet & Gynecol., Isesaki Municipal Hosp., Gunma), Yutaka Suzuki (Dept. Pathol. ,Isesaki Municipal Hosp., Gunma), and Takeshi Matsuda (Dept. Anatomy, Toyama Med & Pharmaceut., Univ., Toyama)

Prenatal diagnosis of Potter's type I cystic kidney disease (autosomal recessive form of infantile polycystic kidney) was performed in 2 siblings by ultrasonographic examination. Case 1. A girl was diagnosed at 36 weeks of gestation as Potter's type I cystic kidney disease by ultrasonographic examination. Fetal bladder was not detected and marked enlargement of the kidneys was found. The longitudinal and transverse diameters of the kidneys were 9.6 x 4.0 cm in the right, and 9.1 x 4.0 cm in the left, respectively, and the texture of the kidneys was hyperechogenic. She died about 4 hours after birth, and autopsy revealed polycystic kidneys(right 200g, left 190g), with accompanying hepatic fibrosis and bile duct proliferation, and pulmonary hypoplasia. Case2. A girl(younger sister of case 1) was found to be without Potter's type I cystic kidney disease by serial ultrasonographic examinations from 30 weeks of gestation to 5 months after birth.

A 23

A CASE WITH 3(p25-pter) TRISOMY DIAGNOSED AFTER THE CHROMOSOME ANALYSIS OF THE MOTHER FOR THE REPEATED ABORTION. Shozo TAMURA, Yuko SHIRAHATA, Mizuho TAKADA (Dept. Obst. & Gyenc., Sch. Med., Keio Univ., Tokyo), Yukari YANAGI (Keio Health Counseling Center, Tokyo), Yoshiyuki HIRAISHI (Dept. Microbiol., Sch. Med., Keio Univ. Tokyo), Masaaki ITO (Inagi City Hosp, Tokyo)

The patient, 10 year old girl, has shown severe maldevelopment and mental retardation strongly suggesting the chromosomal abnormality. However it could not be found in spite of examinations at two other hospitals respectively. After the first visit of her mother to our hospital the chromosome analysis was carried out for the mother because of spontaneous abortion repeated twice and revealed the balanced translocation of t(2;3)(q37;p25). After our efforts of prevailing on her to need further examination once more, the chromosome analysis of patient was carried out and revealed the abnormal karyotype of 46,xx,-2, der(2), rcp(2;3)(q37;p25) mat.

The clinical features are similar to those of 3p trisomy syndrome reported up to now, e.g. square-shaped face, prominent cheek, down-turned corners of mouth, micrognathia, mental retardation etc.

A 25

A CYTOGENETIC STUDY OF SPONTANEOUS ABORTIONS WITH DIRECT ANALYSIS OF CHORIONIC VILLI. Michiko OHNO, Tomoko FUNATO, Noriko YABE, Kyoko YOSHIHARA, Akira MATSUNOBU, Tohru MAEDA (Kitasato University Hospital, Sagamihara, Kanagawa)

A cytogenetic investigation of spontaneous abortion necessitates long-term cell culture from the product of conception for several weeks to obtain a final result. Direct preparations have great advantages in that results can be obtained relatively rapidly and maternal cell contaminations are minimal. We report our experience with 102 cases of spontaneous abortions chromosomally analyzed with direct method on chorionic villi. The typical chorionic villi were separated from the decidua under a dissecting microscope. One hundred and two of the 108 samples were successfully karyotyped(94.4%). Abnormal karyotypes were detected in 69 cases(67.6%). Autosomal trisomy was the predominant abnormality(69.6%), followed by monosomy X (14.5%), structural anomalies(8.7%), and triploidy(7.2%). One case with hydatidiform mole showed the similar Q-band polymorphism in several sets of homologous chromosome. This simple and rapid method is considered to be useful in evaluating the cause of early fetal loss.

A 26

A NEW METHOD FOR OBTAINING HIGH-RESOLUTION BANDED CHROMOSOME PREPARATION FROM CHORIONIC VILLUS SAMPLE. DENG Han-Xiang, LI Lu-Yun, XIA Jia-Hui (Dept. Med. Genet., Hunan Med. Univ., Changsha, China), Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

A new method for obtaining high-resolution banded chromosome preparations from chorionic villus samples (CVS) is described, in which a synchronization cell-culture technique with the use of thymidine is introduced. After culturing a suspension of chorionic villus cells in a medium supplemented with 0.3 mg/ml thymidine for 13-17 hrs, cells were washed twice, and recultured for further 8 hrs. The cells were then treated with 10 μg/ml ethidium bromide or 1 μg/ml actinomycin D for 1 hr and subsequently with 0.01 μg/ml Colcemid for 30 min. The average mitotic index obtained was more than 60 mitoses/mg CVS, and more than 60% of mitoses proceeded to the prometaphase, the later prometaphase stage, or in between. The application of this method for prenatal diagnoses in four CVS in the first trimester pregnancies proved that it is a simple, practical and efficient diagnostic method.

A 27

Relative Reproductive Fitness of Haemophilia. Masao KANAMORI (The Inst. Public Health), Kazuo HAYAKAWA (Kinki Univ.), Kiyotaro KONDO (Hokkaido Univ.), Stephanie SHERMAN and Newton MORTON (Univ. Southampton)

Relative fitness of carriers as a function of fertility, survival, and generation time in pedigree under incomplete ascertainment was estimated by stepwise regression analysis. The basic definition is W=BM/T, where W= relative reproductive fitness of carriers and controls; B=ratio of live births to carriers and controls; M=ratio of survival of live births from carriers and controls; T=ratio of the mean ages of carriers and controls at the time of a live birth (Morton). To estimate B, M and T, we calculate a weighted least squares regression of the type, $y=s/L=a+cX$, where X is the carrier status as a binary variable (0,1) and the weight is L on the assumption of a Poisson distribution of s live births. Carriers are 173 confirmed casas of haemophilia found on the lists of the four hospital haematology laboratories/clinics who were born in South Australia since 1905. Survival time was also estimated by life-table method. We can estimate the B, M, T, (B=.68, M=.94, T=.94) and The relative reproductive fitness of carriers and controls is 0.7056 ± 1408. There is no significant shift in segregation frequency. Implications of these results for population dynamics of structural rearrangements are discussed.

A 28

POPULATION GENETIC STUDY IN ISOLATED COMMUNITIES: Ⅶ .GENETIC POLYMORPHISM IN MIYAMACHO, FUKUI PREF. Masao NAKANAGA, Akira TOKUDA, Masaki DOUCHIN, Shigeaki NAKAZAKI, Kazuhiro KIYOSAWA, Motozumi NOMURA, Mikio HIRAYAMA, Yoshihiro KOHLI, Norio FUJIKI, (Dept. Inter. Med., Fukui Med. Sch., Fukui) and Kazuo MANO (Nagoya 1st Red Cross Hosp., Nagoya)

For over 30 years we have investigated the biological effects of inbreeding and genetic polymorphisms in 14 isolated communities in Japan. This time, genetic and medical survey was completed in 2 isolated communities of Miyamacho, Fukui Prefecture, where have 876 inhabitants in 271 households. According to Koseki recods checking, there revealed 0.005623, 17.3% and 7.2% as the mean inbreeding coefficient, consanguinity rate and first cousin marriage rate, respectively. The medical survey done in 516 of 876 inhabitants (58.9%) did not reveal any cluster of specific genetic diseases or congenital malfomations.

The gene frequencies of many polymorphic traits were culculuted I*A=0.300,I*B=0.172, I*O=0.528 on blood type, Hp*1=0.236, Hp*2=0.764; Tf*Cl=0.797, Tf*C2=0.198, Tf*V=0.005; Gc*1F=0.429, Gc*1S=0.308, Gc*2=0.225, Gc*V=0.038 on serum protein types and P*a=0.231, P*b=0.769; EsD*1=0.643, EsD*2=0.352, EsD*V=0.005 on red cell enzyme types. Variant types of each polymorphism were TfCI-D, Gc1F-1A3,Gc1S-1pA3,Gc1S-1A2 and EsD7-1.

A 29

GENETIC ANALYSIS OF FAMILIAL MOTOR NEURON DISEASES. Kiyotaro KONDO (Department of Public Health, Hokkaido University, Sapporo)

World pedigree reports of familial motor neuron diseases (MND) involving autopsied cases were divided by pathology into two groups ;classical cases and cases with posterior column degeneration. Clinically, latter cases were characterized by 1) 1:1 sex ratio, 2) early onset, 3) onset in the lower limbs, 4) less frequent bulbar and pyramidal signs. Classical cases were identical with sporadic cases. Cases with posterior column degeneration are nearly always familial, and segregation analysis showed a clear-cut dominant inheritance. Classical cases show positive family history in only 1-2% and the family patternswere compatible with a multifactorial inheritance with a relatively low heritability. Familial MND contains at least two distinct entities.

A 30

MORTALITY RATE OF HUNCHINGTON DISEASE IN JAPAN: SECULAR TRENDS, MARITAL STATUS, and GEOGRAPHICAL VARIATIIONS. Yoko Imaizumi (Institute of Population Problems, Ministry of Health and Welfare, Tokyo)

The death rate from Huntington disease (HD) in Japan was analyzed using Japanese vital statistics for 1969-1985. There was no significant change in the HD death rate over the years. The overall death rate per million population was 0.15 for both sexes. As for marital status, a quarter of the HD deaths was the single group for both sexes. There were remarkable differences in the HD death rates for each sex among the four marital categories. The geographical variations in the HD death rate were observed with the highest death rate in Tokushima prefecture (1.03). The mean age at death in HD was nearly constant during the period, and overall mean age at death was 48 years for both sexes, which value was eight years shorter than that in South Wales.

A 31

ASSIGNMENT OF THE APOLIPOPROTEIN A1 GENE TO 11q23 SUGGESTED BY A CASE WITH 11q- SYNDROME. Tadao ARINAMI, Kimiko KOBAYASHI, Yasuko YAMANOUCHI, Hideo HAMAGUCHI (Dept. Hum. Genet. Tsukuba Univ. Ibaraki)

Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoprotein (HDL) and a cofactor for lecithin:cholesterol acyltransferase. The gene coding for apoA-I has been known to be clustered with the genes for apolipoproteins C-III (apoC-III) and A-IV (apoA-IV) within a 15-kilobase (kb) (apoA-I-apoC-III-apoA-IV gene cluster (Karathanasis 1985). This gene cluster has been mapped to the distal portion of the long arm of human chromosome 11 q23-qter (HGM 9) by hybrid analysis (Glaser et al. 1987) and in situ hybridization (Sparkes et al. 1987).

In order to make more precise localization of this gene cluster, we examined restriction fragment length polymorphism (RFLP) genetypes at the apoA-I locus in a boy with 11q- syndrome (46,XY, del (11)(q23.3)) using a genomic apoA-I gene probe. He was proved to be heterozygous for the *Xmn*I RFLP, indicating that 11q24-qter is excluded for the location of the apoA-I gene. Thus the location of the apolipoprotein A-I gene has been more specifically assigned to 11q23.

A 32

THE GENE CODING β-HEXOSAMINIDASE A (HEXA) α-SUBUNIT HAS BEEN ASSIGNED TO 15q23-q24 BY HIGH-RESOLUTION IN SITU HYBRIDIZATION. Kaoru TAKEDA, Katsuya YAMAMOTO, Yoshitsugu YAMAMOTO, Hisashi HAGIWARA, Hiroshi NAKAI, Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai), and Wakae MURAMATSU (Dept. Labor. Med., Tohoku Univ., Sendai)

Tay-Sachs' disease results from the mutation of HEXA α-subunit. Using high-resolution in situ hybridization, we tried to determine the finer gene locus. Materials and Methods: PHA-stimulated normal human male lymphocytes were incuvated. BrdU was added and the block was released by change to thymidine-enriched medium. After harvest without colcemid chromosome preparations were perfomed and pretreated by RNase. The cDNA probe, pβHα-5 insert coding the entire sequences of HEXA α-subunit, was ^{3}H-labeled within $1\text{-}4\times10^{7}$ cpm/mcg of cDNA by nick translation. The labeled cDNA probe and chromosomal DNA were denatured, and molecular hybrids were formed. After washing and antoradiography the slides were stained by Hoechst 33258-UV-Giemsa. Results: Total 227 grains within 115 cells were analysed. The chromosomal region 15q23-q24 had 27 grains, corresponding to 11.9% of the total grains and to 77.1% of the grains on chromosome 15. Although the SRO (shortest region of overlap) of HEXA α-subunit has been 15q22-q25.1 by somatic cell hybrid strategy etc., we could assign the gene locus to the narrower region 15q23-q24.

A 33

CHROMOSOMAL LOCALIZATION OF α-FUCOSIDASE GENE ON THE REGION OF 1p34.3 BY IN SITU HYBRIDIZATION. Hiroshi NAKAI, Kaoru TAKEDA, Katsuya YAMAMOTO, Yoshitsugu YAMAMOTO, Hisashi HAGIWARA, Wakae MURAMATSU and Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai)

A cDNA coding for humanα-L-fucosidase 1(FUCA1) was assigned on a human chromosome 1p34.3 region by in situ hybridization. The probe(λAF3) was isolated from a human hepatoma cDNA library by de Wet et al. It is 1,058 bp in length and encodes a polypeptide fragment of 347 amino acids in length. In situ hybridization was done following to the method of Zabel et al.(1983). Sample probe was labeled with tritium dCTP, dATP, TTP and cold dGTP by nick translation. After micro-autoradiography and G-staining with Hoechst-33258, UV exposure and Giemsa solution, silver grains were protted on a chromosome sheet. Four-hundred fifty eight grains were observed in 264 prometaphases of human lymphocyte stimulated with PHA. 101 grains were on chromosome 1 and 35 grains on the region 1p34.1-.3. This number is 7.7% of total grains, and 32.1% of grains on chromosome 1. A pseudogene on chromosome 2 (FUCAL1) did not make any peaks of grain on the chromosome.

A 34

染色体移入法による ataxia-telangiectasia (D group) 原因遺伝子のマッピング．
押村光雄[1]・小松賢志[2]・児玉清司[2]・奥村寛[2] ([1]神奈川がんセ・研・細胞遺伝，[2]長崎大・原医研・放射線）．CHROMOSOME ASSIGNMENT BY CHROMOSOME TRANSFER, OF AN ATAXIA-TELANGIECTAXIA GENE IN COMPLEMENTATION GROUP D：
Mitsuo Oshimura[1], Kenshi Komatsu[2], Seiji Kodama[2] and Yutaka Okumura[2] ([1]Lab. Cytogenet., Kanagawa Cancer Center Res. Inst., Yokohama; [2]Dept. Rad. Biophys., Nagasaki Univ., Nagasaki)

ataxia-telangiectasia（ＡＴ）は常染色体劣性遺伝病であり，ＡＴ患者から得られた細胞は放射線致死高感受性である。最近，Gatti らはＡグループ相補群のＡＴ患者(AT-A)家系の遺伝子連関分析により，染色体 11q22-23 にその変異遺伝子が存在することを示した（Nature 336：577-580，1988)。

今回，我々は，ＳＶ４０によって形質転換した遺伝子相補群ＤのＡＴ細胞（AT5BIVA）にpSV2neo 遺伝子で標識した正常ヒト線維芽細胞由来の染色体を微小核細胞融合法により移入し，放射線照射による致死率を指標として，ＡＴ－Ｄの変異遺伝子の存在する染色体の同定を試みた。その結果，１２番染色体移入クローンにおける放射線致死感受性は親細胞 AT5BIVA と同様であったが，１１番染色体移入クローンでは正常線維芽細胞(MRC5)と同様の感受性レベルまで回復した。

以上のことは，ＡＴのＤグループ相補群の変異遺伝子もＡグループと同様に，１１番染色体上に存在することを示唆する。

A 35

CHROMOSOME ASSIGNMENT OF HUMAN CARDIAC MYOSIN HEAVY CHAIN GENE TO CHROMOSOME 14q11.2-q13.
YOSHIDA, M.C., MATSUOKA, R., TAKAO, A., and KANDA, N. (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo; Dept. of Pediat. Cardiology, Heart Inst. Japan, Dept. of Anat., Tokyo Women's Med. College, Tokyo)

The human cardiac alpha- and beta-myosin genes, MYH6 and MYH7, were isolated from human genomic cDNA clones, using two rat cardiac pCMH26: alpha-MYH type, and pCMHC 5:beta-MYH type as probes. These two genes were mapped to chromosome 14cen-q13 by analysis of human fibroblastic lines carrying partial deletion or duplication of chromosome 14. The localization of the two genes was substantially confirmed by Southern blot analysis of human-mouse and human hamster hybrid cells and by in situ hybridization which showed a significant silver grain distribution on 14q11.2-q13.

A 36

HUMAN TYPE II COLLAGEN GENE (COL2A1) ASSIGNED TO CHROMOSOME 12q13.11-q13.12 BY NONISOTOPIC IN SITU HYBRIDIZATION. Ei-ichi TAKAHASHI, Tadaaki HORI (Div. Genet., Natl. Inst. Radiol. Sci., Chiba), Jeanne B. LAWRENCE, John McNEIL, Robert SINGER (Dept. Cell Biol., Univ. Mass., Massachusetts), Peter O'CONNELL, Mark LEPPERT and Ray WHITE (Howard Hughes Med. Inst., Univ. Utah, Utah)

We have made a regional assignment of COL2A1 on human chromosome 12 by nonisotopic in situ hybridization with biotinylated DNA probe. For the precise mapping, we have developed the detection system of fluorescent signals on R-banded chromosome stained with propidium iodide. The precise localization was mapped to the band 12q13.11-q13.12. This was in agreement with the mapping by isotopic in situ hybridization technique (12q13.1-q13.2), but not the result of Southern hybridization analysis using somatic cell hybrids (12q14.3). This was further confirmed by using the expression of rare fragile site at 12q13.1, fra(12)-(q13.1) (from Dr. Sutherland). The signal was located at the distal part of the centric counterpart of fra(12)(q13.1).

A 37

A NEW INHERITED DISEASE WITH OPHTALMOPLEGIA, HEARING LOSS, ATAXIA AND SENSORY POLYNEUROPATHY WITH NORMAL MITOCHONDRIAL RESPIRATORY CHAIN ENZYMES. Helena PIHKO, Tuula KOSKINEN, Anna MAJANDER and Pirkko SANTAVUORI (Children's Hospital, Univ. Central Hospital, Helsinki, Finland)

17 patients (9F/8M) from 11 families were investigated for a new disease manifesting as a rapidly progressing encephalopathy during second year of life in infants with normal early development. The ability to walk and speak is lost, ataxia, hypotonia and athetoid movements appear. Ophtalmoplegia and severe sensory hearing loss develop. After the initial deterioration the children regain their interest in the surroundings and mental development continues if adequate communication is provided. Some regain the ability to walk, but most patients are in the wheelchair by the age of 10. The progression of the disease is slow, the oldest patient is 29. The pathophysiology of the disease is unknown. Laboratory tests, including mitochondrial respiratory chain enzymes have been normal. Sensory polyneuropathy can be detected electrophysiologically and by histological changes.

A 38

A STUDY OF SEGREGATION IN THE TRANSLOCATION HETEROZYGOTES. Tamiko SHINOHARA, Haruyoshi URANO, Genichi NOZUE(Japan Red Cross Med Center, Tokyo)and Akira TONOMURA(Med. Res. Inst., Tokyo Med. Dent. Univ.,Tokyo)

During our current survey of chromosome in families with various kinds of translocation heterozygotes, we have found that out of a total of 67 children born to 18 heterozygotes, 29.9% had a normal chromosome complement, 11.9% had a balanced translocation, 19.4% had an unbalanced type and 38.8% of the total pregnances were aborted. The frequency of abortions in these families is highly excessive, as compared with the figure for spontaneous abortions in the general population. In order to examine this peculiar phenomenon, the families were arbitrarily grouped according to the times of abortions. The frequency of abortions was extremely higher in the group with abortions more than in the lower abortion group, while the children having an unbalanced complement were much lower in the higher group. Based on these differences between two groups, the lengths of the excess and deficient segments involved in a translocation were measured. The mean extra length of the liveborns with an unbalanced complement showed almostly equivalent to that of the extra 21 chromosome in Down syndrome. The pattern of segregation in a translocation heterozygote is therefore probably depend not only on the position and frequency of the chiasmata but also the length of the pairing segments.

A 39

SIXTY EIGHT CASES OF CHROMOSOME ANOMALIES DETECTED BY AMNIOCENTESIS. Kodo SATO, Yoshiko MORITA, Fumiyoshi KAYAMA, Noriko TAKANO (Dept. Obstet.Gynecol.,Toranomon Hospital,Tokyo) and Masumi OGAWA (Dept. Hematol.,Toranomon Hospital,Tokyo)

Sixty eight cases of chromosome anomalies detected by amniocentesis at Toranomon Hospital between April,1986 and July,1989 were analyzed and discussed. A total of 1000 amniocentesis was carried out during the same period. The cases with chromosome anomalies included 13 cases of trisomy, 2 of 45,XO, 2 of marker chromosome, 2 of imbalanced translocation, 10 of balanced translocation, 4 of mosaics, 14 of inversion 9, 1 of inversion 11 and others. In 10 cases, blood chromosome analysis was carried out for the parents following amniocentesis in order to detect the origin of anomalies observed in the fetuses. Among the 10 cases, there were 5 cases in whom the same chromosome anomalies were found for either of the parents. And these parents decided to continue their pregnancies. Thus it is suggested to be important to maintain a system where a rapid blood chromosome analysis is available at any time for the prenatal diagnosis unit.

A 40

THE FREQUENCY OF DOWN SYNDROME IN PRENATAL DIAGNOSES. Akira TONOMURA (Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo) and Tamiko SHINOHARA (Japan Red Cross Med. Center, Tokyo)

Recently prenatal cytogenetic diagnoses are increased rapidly in Japan, since the risk of Down syndrome increase with advanced maternal age. In general, it is considered that the frequency of Down syndrome in prenatal diagnoses is higher than that predicted from postnatal studies, because some fraction of Down syndrome conceptions detected at amniocentesis terminates in late fetal death. In order to examine the difference between rates of Down syndrome in prenatal studies and in live births, the data by single-year interval from 2 sources were provided for mothers of 35 years and older. This rate is more than double the rate in New York study of live births to women of almost the same ages. Since the data of risks for Down syndrome in live births were not available in Japanese population, the risks by single-year interval were used from the risks combined with 4 reports by Thompson (1980). For comparison, risks were computed using stated age plus 5 months, because a woman having amniocentesis will be approximately 5 months older at delivery. The number of trisomic fetuses detected was somewhat higher than that expected for mother age 35 - 45, but not statistically significant between two series, because the sample of women examined is probably too small to be informative.

A 41

核家族資料による遺伝標識と病気の連鎖分析：安田徳一[1]・五条堀孝[2]（[1]放医研・遺伝，[2]遺伝研・進化）. FIRST : A COMPUTER PROGRAM FOR LINKAGE ANALYSIS OF RFLPs AND GENETIC DISEASE WITH NUCLEAR FAMILY DATA : Norikazu YASUDA[1] and Takashi GOJOBORI[2] ([1]Div. Genet., Natl. Inst. Radiol. Sci., Chiba ; [2]Dept. Pop. Genet., Natl Inst. Genet., Mishima)

DNA多型を指標とする連鎖分析を中心として、そのアルゴリズム解析とコンピュータプログラムの開発研究を行い、その解析の応用として、家族集積性が非メンデル性である疾患に主要効果を示す遺伝子のマッピングを試みた。

核家族(両親とその子供)を調査資料の単位とし、各家族員は健康か病気の２表現型のいずれかとする。標識となるRFLPsやHLAには相互優性の複対立遺伝子がある。ベイズの定理により各核家族のロッド得点を求め、その総計が 3以上なら連鎖あり、-2以下なら連鎖なし、その他では資料不足とする。この際組換え率の男女差、病気の浸透率、主効果遺伝子の頻度、標識遺伝子の頻度をパラメータとして考慮した。RFLPs, HLAはあらかじめ染色体地図上の位置がわかっているので、これから病気の発症に主効果を表す遺伝子がマッピングされる。基本原理(first principle)によるこの方法は確認法による偏りを定式化の過程で取り込んでいるため、Mortonの Z 得点法のように結婚型で違う偏りの補正をする必要がない利点がある。調査資料を核家族の２世代に限定した欠点はあるが、多くのRFLPsをスクリーニングする最初の段階として有用である。PC9801で実行可能なプログラムＦＩＲＳＴを開発した。

A 42

THE INCIDENCE OF CONGENITAL ANOMALIES IN INTRAUTERINE GROWTH RETARDATION.

Fumio TAKADA, Kiyoshi IMAIZUMI, Yoshikazu KUROKI(Div.Med. Genet.), and Akiko Gotoh(Dept.Neonatol, Kanagawa Child. Med. Cent., Kanagawa)

The relationship between congenital anomalies(CAs) and intrauterine growth retardation(IUGR) was analysed using data of retrospective study of the neonatal admission records in Kanagawa children's medical center. In all 2742 infants, 416 infants with IUGR were ascertained. The frequency was 15.2%. And 32% of them suffered from CAs. The value was 4 times as high as that(7.9%) of general neonate population. IUGR grade classification was made by gestation and severity of IUGR. Chromosome aberrations were more frequentry observed in sever IUGR group. No Menderian inheritance diseases were in preterm group. The proportion of 18 trisomy was extremely high in chromosome aberration group. No sex chromosome aberrations were ascertained. In IUGR group, further prospective studies of CAs, including chromosome analysis of all IUGR cases are necessary to clarify the prevalences of genetic diseases in IUGR population.

A 43

THE OUTCOME OF PREGNANCY IN KANAGAWA AFTER THE CHERNOBYL ACCIDENT.

Yoshikazu KUROKI, Hiroshi KONISHI, Fumio TAKADA, Kiyoshi IMAIZUMI(Div. Med. Genet, Kanagawa Child. Med. Cent., Kanagawa) and Toishimasa KATOH (Kanagawa Society Obstet. Gynecol. Kanagawa)

The radioactive plume released from the Chernobyl accident on April 26, 1986, reached Japan within one week after the accident and induced radioactive contamination over the country. The total deposition of ^{137}Cs in the first one month after the accident was counted as 222 Bq/m^2, 2,200 times as high as that prior to the accident. The annual effective dose equivalent to Japanese population due to radioactive fallout from the accident was assessed to be $5x10^{-6}$ Sv/y(NIR-M 68). This dose corresponds to only 0.2 % of that of 2.4 mSv/y from natural radiation sources. Thus it is well below the teratogenic or mutagenic threshold and is unlikely to increase the incidence of malformations. Nevertheless, we studied the possible teratogenic or mutagenic impacts of the accident based on Kanagawa Birth Defects Monitoring data. There were no significant differences in the incidence of malformations, stillbirth rate, and sex ratio. Especially, the analysis of 3 sentinel phenotypes revealed no measurable changes. The results support other studies in Europe.

A 44

PARENTAL AGES AND THE GERMINAL MUTATIONS AT THE RB LOCUS. Ei MATSUNAGA (Natl. Inst. Genet., Mishima), Kensei MINODA (Teikyo Univ. Ichihara Hosp., Ichihara), Masao S. SASAKI (Rad. Biol. Center, Kyoto Univ., Kyoto)

Statistical analysis was made of the parental ages for 225 patients with sporadic bilateral retinoblastoma, which can be assumed to have arisen from germinal mutations. These patients were born in the periods from 1965 to 1968 and from 1975 to 1982, for which the vital statistics data provide both paternal and maternal age distributions for all legitimate live-births in Japan. The mean paternal age of the patients was 30.2 years, which was close to the 30.1 years for the control population as adjusted by the year of birth of the patients; the mean maternal age was 27.3 years in both patients and controls. Moreover, data on parental ages for ten cases of sporadic retinoblastoma associated with either deletion or translocation involving 13_q14 that was identified as of paternal origin were analyzed. The mean paternal and maternal ages of those cases were again close to the controls. These results suggest that parental exposure to ionizing radiation or chemical mutagens, the effect of which is accumulated with advancing age, does not play a major role in the production of germinal mutations at the RB locus.

A 46

SITE POLYMORPHISM WITHIN 3'-"bcr" 1.2KB HindIII/BglII FRAGMENT IN THE JAPANESE INDIVIDUALS

Masayoshi TSUTSUMI Kyoko KATO, Yasunobu YOKOYAMA, Akira YOSHIDA, Susumu SAITO, Noriko TOBISHIMA, Takanobu SAIGO, Kazumasa HIKIJI, Yutaka TSUKADA, Masayoshi TAKAHASHI(Dept. Genet. Res. Lab. SRL Inc. Tokyo)

It is well known that the Philadelphia chromosome, resuluting from the 9;22 translocation, is observed in more than 95% patients of Chronic Myelocytic Leukemia(CML), and the Ph-positive CML patients consistently show a rearrangement in the 5.8kb breakpoint cluster region(bcr). In this paper, we present the molecular biological studies for eight cases of site polymorphism within 3'-"bcr" probe in the Japanese individuals. We performed the southern blot analysis for 750 patients of CML or MPDS and 67 normal individuals with 1.2kb HindIII/BglII 3'probe(Oncor Inc.), and found the following results. 1)The two unusual bands, 2.0 and 1.4kb digested from 3.4kb with BamHI restriction enzyime was observed in eight patients. 2)The same two bands were observed in high molecular weight DNA of one patient extracted from fibroblast. 3)In 3 CML patients, the rearrangement was always observed within opposite allele of this unusual bands. 4)However, the unusual two bands with BamHI digetion were not find in 67 normal individuals. In conclusion, these unusual two bands are a site polymorphism from the preceding results conformed by the Hardy-Weinberg Law. The BamHI site in 3'-"bcr" probe is oriented in 1.4kb 5'BamHI site.

A 47

ANALYSIS OF CHIMERIC bcr-abl m-RNA IN CHRONIC MYELOCYTIC LEUKEMIA BY MEANS OF PCR METHOD .Kimio TANAKA,Miho TAKECHI,Nanao KAMADA (Dept.Hematol.,Inst.Nuclear Medicine & Biol.,Hiroshima Univ.,Hiroshima)

Gene alteration of fused bcr-abl chimeric gene and it's expression were studied by PCR method and Southern blotting in 36 Phl chromosome positive CML patients .Two types of chimeric m-RNA were expressed in the junction of bcr-abl gene. A strong correlation between breakpoint region in bcr and types of expression of the chimeric m-RNA was observed.The 8 patient with breakpoint at more 5'side of bcr had expression of bcr2-abl alone and 26 patients with breakpoint at 3'side of bcr had bothtypes of bcr2-abl and bcr3-abl m-RNA simultaneosly and/or bcr3-abl m-RNA A only.The expression of bcr3-abl m-RNA usually expressed dominantly.In other two patients who had breakp oint outside of bcr and had in 5'side of bcr,the fused m-RNA were not detected by PCR method .In only two of eleven patients who were studied sequencially at different stages,alteration of types of the chimeric gene was observed in only two patients .The studies on the patients treated with bone marrow transplantation and interpheron therapy confirmed us that the PCR method is extremely sensitive and reliable to detect minimum resudual cells for diagnosis.

A 48

TRISOMY 9 IN Ph-NEGATIVE CHRONIC EOSINOPHILIC LEUKEMIA. Yoshio NISHIZAWA, Miyako YAMAMOTO, Mayumi YAMADA, Noriko YAKU (Dep. Gen., Nishizawa Clinic, Osaka) and Yasuko NISHIZAWA (Dep. Gen., Nishizawa Clinic, Osaka and Dep. Path., Osaka Univ., Osaka)

The results of cytogenetic analysis of bone marrow and peripheral blood cells in a 82 years-old male with chronic eosinophilic leukamia (CEL) are reported. He came to our Clinic because of bilateral leg pain. Atopic dermatitis in whole body and bronchial asthma had appeared on him for 2 years. He had a severe anemia (myelodysplastic state) due to CEL. Diagnosis of CEL was done by the Ultmann's criteria, blood picture, increase of serum vit. B12 and leukemia cell markers. His peripheral blood examination showed a marked leukocytosis (21,960/mm^3) with 87.6% eosinophils. The bone marrow smear also showed an increase of eosinophils (84.3%) with mature and immature cellular characteristic. The chromosomal studies revealed the presence of trisomy 9 in these malignant cells, the other cells (lymphocytes etc.) being normal. So far, trisomy 9 has been described for four cases of EL. However, these malignant cells had additional cytogenetic abnormalities. This paper is the first report for an EL case with only trisomy 9 . Cytogenetic studies for EL by others are also discussed. By use of the established cell line from these EL cells, oncogenes of EL cells and their expression are now under study.

A 49

CLINICAL AND HEMATOLOGIC CHARACTERISTICS OF ACUTE LEUKEMIA AND LYMPHOMA WITH t(11;19)(q23;p13) OR t(1;19)(q23;p13)

Yasuhiko KANEKO, Hirofumi KOBAYASHI, Nobuo MASEKI, Chieko HOMMA, Masaharu SAKURAI (Saitama Cancer Center, Saitama)

In a chromosome study of leukemia/lymphoma we found t(11;19)(q23;p13) in 8 patients and t(1;19)(q23;p13) in 9. Both translocations had a common 19p13 breakpoint. The median age of the patients with t(11;19) was younger than that of the patients with t(1;19)(2 yrs vs 7 yrs). The median leukocyte count of the patients with t(11;19) was higher than that of patients with t(1;19)(111,400/μl vs 9,300/μl). t(11;19) was found in 5 patients with acute lymphoblastic leukemia (ALL), 1 with acute myelomonocytic leukemia, and 2 with malignant lymphoma (ML), and t(1;19) in 8 with ALL and 1 with ML. Leukemic cells with t(1;19) had more mature precursor B cell phenotype than those with t(11;19). The median survival of the patients with t(11;19) was longer than those with t(1;19). The genes located in 19p13 involving in t(11;19) or t(1;19) may be different if we consider the clear phenotypic differences between the 2 types of leukemias/lymphomas. Alternatively, the same gene located in 19p13 may be involved in the 2 translocations, and the different phenotypes may be caused by the other gene located in 11q23 or 1q23.

A 50

PHYSICAL MAPPING OF THE MEN2A REGION BY PULSED-FIELD GEL ELECTROPHORESIS.

Norifumi TANAKA, Masayuki YAMAMOTO, Makoto OKAZAKI, Katsu KARAKAWA, Eisei SHIN, Tetsuro KOBAYASHI, Shin-ichiro TAKAI (2nd Dept. Surg.,Osaka Univ. Sch. Med., Osaka), Jun NAKURA and Tetsuro MIKI (Dept. Geriat. Med., Osaka Univ. Med. Sch., Osaka)

The gene for multiple endocrine neoplasia type 2A(MEN2A),an autosomal dominant disease characterized by medullary thyroid carcinoma and pheochromocytoma, has been assigned to the pericentmeric region of chromosome 10 by the linkage study. MEN2A locus is closely linked to RBP3(interstitial retinol-binding protein 3) and pMCK2 (D10S15). We have constructed a rare-cutter restriction enzyme map around RBP3 and pMCK2 by pulsed-field gel electrophoresis, which is a powerful tool to determine the long-range restriction map. RBP3 and pMCK2 have been localized within the MluI 160kb fragment. The result that most of rare-cutter restriction sites are localized within the small region suggests the existence of G-C rich island flanking to the RBP3 locus. In some patients with MEN2A, the mutations such as chromosomal deletion were not found in this region. Since RBP3 locus is not close enough to the MEN2A locus, new DNA markers must be isolated from the pericentmeric region of chromosome 10.

A 51

PRECLINICAL DETECTION OF MEN2A GENE CARRIER USING CLOSELY LINKED DNA MARKERS. Masayuki YAMAMOTO, Norifumi TANAKA, Makoto OKAZAKI, Eisei SHIN, Katsu KARAKAWA, Tetsuro KOBAYASHI, Shin-ichiro TAKAI(2nd Dept. Surg., Osaka Univ. Med. Sch., Osaka), Tetsuro MIKI and Toshio Ogiwara (Dept. Geriat. Med., Osaka Univ. Sch. Med., Osaka)

The gene for multiple endocrine neoplasia type 2A(MEN2A) has been assigned to the pericentromeric region of chromosome 10 by linkage analysis. The preclinical detection of gene carriers in MEN2A family using polymorphic DNA markers tightly linked to MEN2A locus is useful for early diagnosis and early treatment of this disease. We have used RBP3 (interstitial retinol-binding protein 3) and FNRB (beta subunit of the human fibronectin receptor) close to the MEN2A gene as probes. The maximum lod scores are 5.96 (Omax=0.00) between MEN2A and RBP3, and 4.91 (Omax=0.1) between MEN2A and FNRB. In one informative family, one female is determined to be gene carrier at less than 1% risk. She is 20 years old now, and careful following up by calcitonin stimulation test should be required. We conclude that DNA-based prediction of MEN2A is an effective procedure.

A 52

ANALYSES OF HUMAN NEUROBLASTOMA DNA USING RFLP MARKERS AT CHROMOSOME 1p FOR POSSIBLE LOSS OF HETEROZYGOSITY. Masao YAMADA, Mieko KATO, Shin-ichi YOKOTA, Atsushi AKANE, Yutaka NAKAHORI, and Yasuo NAKAGOME (National Children's Medical Research Center, Taishido, Setagaya, Tokyo 154)

Partial deletion of the short arm of chromosome 1 (1p31-ter) was frequently observed in human neuroblastomas by cytogenetical analyses. We examined whether loss of heterozygosity (LOH) for chromosome 1p occurred in neuroblastomas using the following probes: RNU1 at 1p36, DNF15S1 at 1p36, FGR at 1p36.2-36.1, D1S57 and MYCL at 1p32. Only three cases of LOH was detected in 27 pairs of normal and tumor DNAs. Since some of the probes were less informative in the Japanese population, we analyzed additional more than 100 tumor DNAs whose normal counterparts were not available, and compared the distribution of genotypes in tumors with those expected from allelic frequencies in the normal population. LOH was, if occurred, only in 10-15 % cases at the MYCL-D1S57-FGR region of the chromosome, which was much less than the frequency observed by cytogenetical analyses. Taken together a recent report by Fong et al. in Proc. Natl. Acad. Sci. USA 86:3753(1989), a discrepancy between cytogenetical and DNA analyses was discussed.

A 53

正常ヒト1番染色体移入により腫瘍形質が抑制されたDT細胞の v-Ki-ras 遺伝子発現とTGF産生について。 波柴弘樹[1]・堀川 泉[1]・鈴木勝雄[2]・山田秀人[1]・押村光雄[1]（神奈川がんセ・研・[1]細胞遺伝，[2]生化）。 NORMAL HUMAN CHROMOSOME 1 SUPPRESSES THE TRANSFORMED PHENOTYPES OF DT CELLS WITHOUT AFFECTING v-Ki-ras GENE EXPRESSION AND TGF PRODUCTION. Hiroki HASHIBA[1], Izumi HORIKAWA[1], Katsuo SUZUKI[2], Hideto YAMADA[1] and Mitsuo OSHIMURA[1] (Lab.[1]Cytogenet. and [2]Biochem., Kanagawa Cancer Center Res. Inst., Yokohama)

正常ヒト染色体移入によるがん細胞の腫瘍形質抑制機序の解明を目的として，Kirsten肉腫ウイルス感染形質転換 NIH/3T3細胞(DT)に，微小核細胞融合法により正常ヒト1番，11番，12番染色体をそれぞれ移入し， in vitro の増殖特性および造腫瘍性への影響を検討した。その結果，1番移入クローンでは，倍加時間と血清要求性がNIH/3T3 細胞と同様になり，足場非依存性増殖も著しく低下したのに対して，11番および12番移入クローンでは，いずれの形質もDT細胞と同様であった。一方，いずれのクローンも造腫瘍性を有したが，1番移入クローンでのみ潜伏期の延長が認められたため，各腫瘍の染色体解析を行ったところ，1番移入クローンの腫瘍では移入染色体が脱落していた。さらに，各クローンにつき v-Ki-ras 遺伝子の発現および増殖因子(TGF)の培地中への産生を検索したが，DT細胞との差は認められなかった。以上の結果より，正常ヒト1番染色体はDT細胞の腫瘍形質を抑制するが，その作用点は v-Ki-ras 遺伝子発現およびTGF産生以降の情報伝達系であろうと考えられる。

A 54

CHROMOSOME ABNORMALITIES IN ADENOMATOUS POLYPS FROM THE PATIENTS WITH FAMILIAL POLYPOSIS COLI (FPC). Mitsuaki A. YOSHIDA[1], Tatsuro IKEUCHI[1], Takeo IWAMA[2], Joji UTSUNOMIYA[4], Isao OKAYASU[3], Akira TONOMURA[1] ([1]Dept. Genet., Med. Res. Inst., [2]Centr. Anal. Polyposis & Intest. Dis., [3]Dept. Pathol. Hosp., Tokyo Med. Dent. Univ., Tokyo, [4]2nd Dept. Surg., Hyogo Coll. Med., Nishinomiya)

Familial polyposis coli (FPC) is a typical cancer predisposing disease and inherited as an autosomal dominant trait. Affected persons have many adenomatous polyps in the colorectum, and if left untreated, the patients are at a high risk for colorectal cancer. Chromosome analyses were successfully performed on short term cultures of 37 individual polyps and of 6 mixed multiple small polyps from 17 patients. All of polyps showed clonal and/or nonclonal chromosome abnormalities in both structure and number. Among these, most frequent abnormality was trisomy #7, which was identified in 14 cultures of polyps. Gains of #12 and #13 were also found in each 5 cultures, and loss of #10 was observed in 8 cultures. Clonal structural abnormalities were less frequent than the numerical abnormalities. However, structural changes involving #1p, #5q and #10q were frequently observed in the present series. These results indicate that the chromosome changes ocurring nonrandomly may be associated with accelerated proliferation and/or malignant transformation of cells in polyp.

A 55

THE ABILITY OF CHROMOSOME 5 TO SUPPRESS THE GROWTH OF A COLONIC CARCINOMA CELL LINE.

Kazunori URABE[1], Senji SHIRASAWA[1], Yasufumi KANEDA[2], Masayuki SASAKI[1], Yuchio YANAGAWA[1], Kenji SUGIO[1], Masao SASAKI[3], Norio WAKE[4], Tsuyoshi UCHIDA[2] and Takehiko SASAZUKI[1] ([1] Dept.Genet., [4]Dept. Repro. Physio. Endocrin., Med. Inst.Bioreg., Kyushu Univ., [2]Inst. Mol. Cell. Bio., Osaka Univ., [3]Radiation Biol. Cent., Kyoto Univ.)

The major gene of familial polyposis coli (FPC) which is an autosomal dominant disease with high risk of colon carcinoma was mapped on chromosome 5q21-22. And allelic losses on chromosome 5 were frequently observed in both sporadic colorectal carcinomas and carcinomas derived from patients with FPC.

In an attempt to examine the role of losses of chromosome 5 in the process of malignant transformation, we introduced normal chromosome 5 into a colon carcinoma cell line, SW620, which lacked a distal region of a long arm of chromosome 5. We obtained several clones and these clones were confirmed by karyotypic and RFLPs analyses to contain the normal chromosome 5 in addition to the chromosomes originally present in the SW620 cells. We observed a measurable and reproducible decrease in the ability of these clones to grow in monolayer compared to the parental cells, although no difference were observed in morphology, tumorigenicity in nude mice, and colny-forming ability in soft agar. These observation suggested that the tumor suppressor gene might locate on chromosome 5.

A 56

GENETIC ANALYSIS OF TYROSINE HYDROXYIASE GENE IN JUVENILE PARKINSONISM.

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We have perfomed linkage study using chromosome 11p markers on 12 patients (8 families) with Juvenile Parkinsonism (JP), which is characterized by an autosomal recessive inheritance, dramatic effect of L-DOPA, juvenile onset and slowly progressive course. We analyzed the posibility of linkage between JP locus and chromosome 11p markers (TH, INS, HRAS1; kind gift from Dr. E.I.Ginns, NIMH) by the homozygosity mapping and the LINKAGE computer program package. Southern blotting analysis showed identical RFLP patterns both in JP patients and controls. In homozygosity mapping, JP patients had the same frequency of homozygosity, compared with control's one. We calculated the pairwise lod score between chromosome 11p markers (TH, INS, HRAS1) and JP at various recombination fractions and the lod score was less than -2 at a recombination fraction theta from 0 to 0.10. Three point likelihood calculations computed with the LINKAGE program showed no linkage between JP and TH within 20cM from the locus defined by TH.

A 57

MITOCHONDRIAL DNA MUTATION IN FAMILIES WITH LEBER'S HEREDITARY OPTIC NEUROPATHY. Makoto YONEDA, Shoji TSUJI, Toyoaki YAMAUCHI, Takashi INUZUKA, Tadashi MIYATAKE (Dept. Neurol., Brain Res. Inst., Niigata Univ. Niigata) Haruki ABE (Dept. Ophthalmol., Niigata Univ., Niigata) Satoshi HORAI (Lab. Hum. Genet., National Inst. Genet., Mishima) Takayuki OZAWA (Dept. Biomed. Chem., Nagoya Univ., Nagoya)

A G-to-A transition at nt. 11778, converting 340th Arg to His in NADH dehydrogenase subunit 4 of mitochondria, has been suggested as the mutation correlated with Leber's hereditary optic neuropathy (LHON). The mutation removes an SfaNI restriction site, which has been found in a few North American and European pedigrees (Wallace et al). We have studied leukocyte mtDNA SfaNI restriction fragment length polymorphism in two Japanese families with LHON and found the identical mutation only in maternal members, including affected patients. Nucleotide sequence analysis of polymerase chain reaction products of patients' mtDNA revealed a G-to-A transition identical to that reported by Wallace et al. This mutation is maternally inherited and be found in patients of different ethnic origin, which strongly support that the identical specific point mutation in mtDNA results in LHON.

A 58

DELETION MUTATIONS OF THE LDL RECEPTOR GENE IN JAPANESE FAMILIAL HYPERCHOLESTEROLEMIA

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Mutations in the gene for LDL receptor give rise to familial hypercholesterolemia (FH). To examine the approximate frequency and features of gross structural rearrangements in the LDL receptor gene in Japanese, we have screened mutant LDL receptor genes from 20 pedigrees with classic heterozygous FH and one pedigree with homozygous FH by Southern blot hybridization, using LDL receptor cDNA fragments as probes. Five different deletion mutations were detected among 22 mutant LDL receptor genes (22.7%); they were characterized by restriction mapping. None of these mutations has previously been reported in Caucasian patients with FH. In three of five deletion mutations, the rearrangements were related to intron 15 of the LDL receptor gene, in which many *Alu* sequences exist. The data suggest that a wide range of molecular heterogeneity exists even in major rearrangements resulting in deletions in the LDL receptor gene, and there is the possibility that a higher frequency of deletion mutations occurs in classic FH than previously suspected.

A 59

PRENATAL DIAGNOSIS OF 21-HYDROXYLASE DEFICIENCY WITH DNA ANALYSIS. Ritsuko ADACHI, Kaoru SUZUMORI, Yoshiaki YAGAMI(Dept. Gyne. Obstet., Nagoya City Univ.), Tatsuro KONDOH, Tadashi MATSUMOTO, Norio NIIKAWA(Dept. Hum. Genet., Nagasaki Univ.)

21-Hydroxylase Deficiency (21-OHD) is the most common error accounting for about 90% of congenital adrenal hyperplasia (CAH) with an incident of 1/15,000 among the Japanese. We report seven 21-OHD families with DNA analysis using the Southern hybridization method. Genomic DNA was extracted from peripheral blood leucocytes of the family members and the chorionic villi of fetuses in the first trimester. The Southern hybridization was performed using the 21-hydroxylase (21-OHase) cDNA and the C4 cDNA as probes. Prenatal diagnosis of three families was performed using chorionic villi. The probands of these three families lacked 3.7kb fragment corresponding to the 21-OHase gene. Three fetuses had 3.7kb fragment and were diagnosed not to be patients. The probands of the other three families showed a normal pattern of the DNA fragments except one family in which the patient was dead. In one family of the three families the RFLP was detected with the enzyme-probe combination of TaqI-the C4 cDNA. The advantage of prenatal diagnosis of 21-OHD with DNA analysis using chorionic villi is that treatment can be initiated as early as possible.

A 60

PRENATAL DIAGNOSIS OF 21-HYDROXYRASE DEFICIENCY USING POLYMERASE CHAIN REACTION. Yoshihiko Noma, Kenji Shima (Dept.Laboratory Med.,Tokushima Univ.,Tokushima), Tetsuro Miki, Jun Nakura, Hiroshi Ikegami, Toshio Ogihara(Dept.Geriatric Med.,Osaka Univ.,Osaka)

Two kinds of DNA analyse have been utilized for the prenatal diagnosis of 21-hydoroxylase(21OHase) deficiency, detection of 21OHase B gene deficiency and RFLPs of Complement4 gene. But 20-30% of the patients can not be diagnosed by these methods. Additional method for diagnosis must be established. To diagnose the desease by the analysis of 21OHase B gene, its fragment was amplified by polymerase chain reaction using oligonucleotid primers specific for B gene. With this method the patient completely deficient of 21-OHase B gene can be diagnosed within several hours, since no 21OHase B gene fragment is amplified. For diagnosis of the patient whose 21OHase B genes are not defficient, RFLPs in the amplified B gene fragment is thought to be useful. Because the sequences of the 2nd intron are most variable between A nad B gene , the RFLPs seam to be frequent in the amplified B fragment containing 2nd intron. To certify the RFLPs, amplified B gene fragments from 10 normal men were digested with several restriction enzymes. 4 polymorphic sites in the amplified fragment were demonstrated by HaeIII, DdeI, and Fnu4HI digestion. 21OHase deficiency can be diagnosed prenatally by means of RFLPs in the amplified B fragments.

A 61

FIBRONECTIN RECEPTOR ON POLYMORPHONUCLEAR LEUKOCYTES IN FAMILIES WITH EHLERS-DANLOS SYNDROME AND OTHER HEREDITARY CONNECTIVE TISSUE DISEASES.
Shinji MIURA, Takuji OHARA, Toshiaki TAKEICHI, Hisaomi KAWAI and Shiro SAITO (The 1st Dep. Int. Med., Tokushima Univ., Tokushima) and Akira SHIRAKAMI (School of Med. Sci., Tokushima Univ., Tokushima)

We analysed fibronectin receptor (FNR) on polymorphonuclear leukocytes in the patients with hereditary connective tissue diseases and their healthy families. In four patients with Ehlers-Danlos syndrome (EDS) type II (family E) and type VI (family M), the number of maximal binding sites (Bmax) of FNR was significantly decreased to 2.2-2.9X10^3 sites/cell (normal range : 6.3±1.5X10^3). The Bmax of their healthy families was normal or slightly decreased to 3.8-5.1X10^3 sites/cell. In a patient with osteogenesis imperfecta type III (family K) the Bmax was significantly decreased to 1.1X10^3 sites/cell. His healthy families showed normal Bmax values. In a patient with Marfan syndrome (family A) the Bmax was decreased to 4.3X10^3 sites/cell. Dissociation constant (Kd) of the FNR was normal in all subjects examined. Some of the healthy members of EDS families had moderately decreased Bmax values, suggesting that they are the carrier of abnormal gene of the disorder. These data suggest that FNR is closely related to the pathogenesis of hereditary connective tissue diseases.

A 62

A SEMIMICRO DIAGNOSTIC METHOD FOR FAMILIAL AMYLOIDOTIC POLYNEUROPATHY BY ISOLATION OF VARIANT TRANSTHYRETIN FROM PLASMA
Yasuyo SUZUKI, Yuji NAKATSUJI, Tomokazu SUZUKI (Department of Clinical Genetics, Medical Institute of Bioregulation, Kyushu University, Beppu)

We previously reported a diagnostic method for type 1 familial amyloidotic polyneuropathy(FAP) based on isolation of variant transthyretin(TTR) from plasma(Suzuki T et al. Neurology 37: 708, 1987). However it had a drawback that 5-10 ml of plasma was needed. In order to improve the diagnostic sensitivity of this test, we adopted an enzyme linked immunosorbent assay(ELISA) in place of immunodiffusion and absorbance for monitoring TTR in chromatographic steps. The detection limit of ELISA for TTR was 50 pg. Consequently ELISA of fractions in reverse phase HPLC of TTR isolated from 0.2 ml of plasma clearly showed two distinct peaks on the elution profile of TTR from the patients, while concomitant peak monitoring by absorbance at 214 nm was scanty to detect it.

The procedure provides a practically useful semimicro method for diagnosing FAP in affected patients and for preclinically testing children of patients with FAP.

A 63

DISTRIBUTION OF AN IMMUNOREACTIVITY TO A MONOCLONAL ANTIBODY AGAINST THE β PROTEIN IN THE SERA OF PATIENTS WITH ALZHEIMER'S DISEASE.
Tomotaka SHINODA, Yoshie KAMETANI (Dept. Chem., Tokyo Metropol. Univ., Tokyo), Kazuo MIYANAGA (Dept. Neuropsychiat., Gunma Univ. Sch. Med., Maebashi) and Kazuso IINUMA (Nat. Child. Hosp., Tokyo)

A monoclonal antibody was raised against a synthetic subpeptide corresponding to residues (N: 1-10, SP01) of the β protein reported to be a major constituent of the barin amyloid of patients with Alzheimer's disease and also of adult Down's syndrome brain amyloid. The monoclonal antibody (aSP01) was shown to have a titer of over 10000 by the ELISA technique; thus it was used for subsequent studies. With this antibody, the level of SP01-RSA conjugate (a control antigen) could be determined in the concentration range of 5-1000 ng/ml in the system. Using the aSP01 and sandwich ELISA technique, crossreactivities in the sera were determined for four different groups, three affected groups(Alzheimer's disease/ senile dementia of Alzheimer's type(AD/SDAT), MID, NOS) and a non-affected group. Whereas a high value in the crossreactivity was observed for AD/SDAT (35.11 ± 7.91 ng/ml), relatively low values were obtained for the latter groups (MID : 16.76 ± 2.58; NOS : 13.28 ± 2.20; non-affected : 14.41 ± 1.94 ng/ml). NO difference in the mean value of the crossreactivity was shown between male and female groups (male : 16.04 ± 3.51, female : 14.99 ± 2.47 ng/ml).

A 64

THE SURVEY OF ABNORMAL HEMOGLOBINS IN TAKAMATSU, FUKUYAMA AND OKAYAMA DISTRICT:
Kazuo HIDAKA[1]; Iwao IUCHI[1]; Hiroko NAKAHARA[1]; Tomoko TAKAGI[1]; Minoru KUWASHIMA[2]and Goro IWAKAWA[3] ([1]Kawasaki Med. Sch., Kurashiki, [2]Kagawa Centl. Hosp., Takamatsu, [3]Natl, Fukuyama Hosp., Fukuyama)

The survey of abnormal Hb(abn.Hb) in Takamatsu, Fukuyama and Okayama district was summarized as follows: I)Takamatsu: Fifty carriers of abn. Hb were detected in 73536 individuals for the past 10 yr. The incidence of abn.Hb was 1/1471=0.07% showing 2 times higher than generally accepted value of the Japanese(1/4000). The details of 50 cases were Hb Takamatsu(24), Hb Ube-2(9), Hb Yusa(3), Hb Mizushi(1), Hb G Szuhu(1), Hb J Bangkok(1) and Hb Camden(1). II)Fukuyama: Twenty eight carriers were in 31419 individuals for the past 4 yr(incidence,1/1122=0.09%). Eighteen cases in 12 families conformed as Hb Ube-2. One case of each of Hb St. Lukes, Hb Fukuyama, Hb Hikara, Hb F-Fukuyama, Hb F-Kotabuki and Hb F-Forest Park was seen. III)Okayama: Four cases were detected in 12678 individuals for the past 1 yr(incidence,1/3169=0.03%). Hb Ube-2(1), Hb Hoshida(1) and HbOkayama-3(tentatively named,1) were detected. Hb Okayama -3(α126Asp→Val) was a new variant demonstrating a high oxygen affinity [$logP_{50}$=0.20(HbA,$logP_{50}$=1.08), Hill'sn=1.64(2.59)]. All the carriers of Th Takamatsu in Japan together with the reports of other laboratories indicated their origen is referred into the inhabitant of Takamatsu area. It is reasonable to think that this gene is harmless, and then not subjected to any natural selection, and become endemic in this localized population.

A 65

NEWLY FOUND NONDELETION TYPE OF HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN (TOYOTA TYPE OF HPFH) IN A JAPANESE FAMILY. Koji SHIMIZU and Hiromi KEINO (Dept. Morph., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Heterocellular nondeletion type of hereditary persistence of fetal hemoglobin (HPFH) has been found in a resident in Toyota, Aichi. Fetal hemoglobin (Hb F) levels were about 15% in the proband, 11% in his mother, 5.3% in his father, and 6.2%in his sister. The percentage of F-cells to total red cells was about 70% in the proband, 56% in his mother, 17% in his father, and 22% in his sister. Their Hb A_2 values were within normal levels (2.0-3.1%). The percentage of $G\gamma$- to total γ-globin chain was about 86% inthe proband, 92% in his mother, 55% in his father, and 53% in his sister. Gene mapping by restriction endonucleases did not show any large deletion in their β-globin gene cluster. The Xmn I site 5' to (at -158 bp of the cap site of) the $G\gamma$-globin gene was +/+ in the proband and his mother, and +/- in his father and sister. It is highly probable that the proband and his mother possess factors as hetertozygous form for both high Hb F and high $G\gamma$ levels, or they are heterozygotes for Toyota type of HPFH. Different unknown factors from them for high Hb F may be present in the father and sister. Further molecular studies at DNA level are now in progress.

A 66

ABNORMAL EXPRESSIONS OF THE BLOOD GROUP A AND H ANTIGNENS ON RED CELLS FROM A CANCER PATIENT. Takasumi MATSUKI, Shun-iti SHIMANO* and Ken FURUKAWA (Dept. Legal Med., Gunma Univ. School of Med., Maebashi, and * Dept. Med., Gunma Cancer Center)

Red cells from 51 years old male patient diagnosed as an acute myelocytic leukemia showed mixed field agglutination to anti-A antibody and no agglutination to anti-B. The free red cells could be separated from A cells by anti-A agglutinin and the proportion of the free cells was about 80 % of total cells. Anti-B and neither anti-A nor anti-H antibodies were found in serum. Saliva contained A and H substances in normal amount. Agglutinability of A red cells to anti-A_1 Dolichos lectin was as weak as that of A_{int} red cells. Agglutinabilities of the separated free red cells to anti-H eel serum and Ulex lectin were 64 to 16 times weaker than that of normal group O red cells. Weaker reaction to Ricinus communis lectin and stronger reaction to Psathyrella velutina lectin of the weak H red cells suggested that galactose residues on non-reducing end of carbohydrate chains of H antigens on the red cells were reduced and N-acetylglucosamine residues were increased compared with those of normal O red cells. Serum N-acetylgalactosaminyl transferase activity which convert O red cells into A red cells was the same as those in sera from normal group A persons. These results suggest that group A_{int} and weak H red cells were presented in the blood of the patient. No difference was found in other blood groups Le(a-b+), MN, P_2 and CDe/cde between components of the mixed red cells except in A and H antigen strength.

A 67

GENETIC POLYMORPHISM OF A GLYCOPROTEIN (GP43) DETECTED IN HUMAN SERUM
Koichiro KISHI, Keiko MIZUTA, Yoko IKEHARA amd Toshihiro YASUDA
(Dept. Legal Med., Fukui Med. Sch., Fukui)

A new and previously undescribed glycoprotein with a molecular weight of 43,000 has been isoleted from human urine. This protein, designated GP43, was co-purified with ribonuclease, which has the same molecular weight, but ribonuclease activity was removed by passage through an affinity column of agarose-5'-(4-amino phenyl phosphoryl) uridine 2'(3') phosphate. GP43 contains about 5.9% neutral sugar, 2.3% hexosamine and 1.6% sialic acid. A rabbit antibody to the purified GP43 reacted with human urine and serum as well as with the purified GP43.

The genetic polymorphism of GP43 was then studied in desialylated human serum samples by urea-polyacrylamide gel isoelectric focusing, followed by immunoblotting with the specific antibody for GP43. Three common phenotypes, designated GP43 1, 1-2, and 2, were easily recognized using this technique, and represented homozygosity or heterozygosity for two autosomal co-dominant alleles, GP43*1 and GP43*2. The frequencies of the GP43*1 and GP43*2 alleles in a studied Japanese population were 0.7687 and 0.2313, respectively (Mizuta et al., Biochem. Genet., Vol. 27, No. 11/12, in press, 1989).

A 68

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN THE JAPANESE POPULATION.
Shin-ichi YOKOTA, Atsushi AKANE, Mieko KATo, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (National Children's Medical Research Center, Taishido, Setagaya, Tokyo 154)

Restriction fragment length polymorphisms (RFLPs) are a powerful form of genetic marker in familial and population analyses for genetic diseases as well as for cancer researches. Almost all the RFLP probes so far available were established after screening of DNA samples isolated from Caucasian. Before use of such probes for analyses in the Japanese population, the detected polymorphisms and allelic frequencies in Japanese should be analyzed to select useful probes. With combination of the results which we reported at the 32nd and 33rd Annual Meeting of Japanese Human Genetics Society, a total of 87 loci for such RFLP probes have been analyzed. We found that a considerable fraction of the RFLPs were specific to ethnic groups.

A 69

POLYMORPHISM OF VNTR (D1S58) LOCUS DETECTED BY THE PCR AMPLIFICATION IN JAPANESE POPULATION. Kentaro KASAI, Harutaka MUKOYAMA (Second Medico-Legal Section, Natl. Res. Inst. of Police Sci., Tokyo), Yusuke NAKAMURA, Raymond L. WHITE (Howard Hughes Med. Inst., Univ. of Utah, Salt Lake City, Utah, USA)

A genetic locus (D1S58, defined by DNA probe pMCT118) that contains a variable number of tandem repeat (VNTR) has been successfully amplified from a very small amount of human genomic DNA by the polymerase chain reaction (PCR). The DNA sequence of the locus was found to consist of a 16-base consensus sequence and flanking sequences. Two ng of human genomic DNA isolated from blood was amplified, and polymorphic bands were detectable by ethidium bromide staining after electrophoresis on polyacrylamide gel. At a high concentration of primers, no significant difference in intensity was found between the larger alleles and smaller alleles, regardless of their sizes. We amplified the MCT118 locus in DNAs isolated from 60 unrelated Japanese subjects. The alleles within this population sample ranged from 387 to 723 base-pairs in length, as expected for a VNTR containing 15 to 36 repeating units of the size present in MCT118. As a pedigree analysis of a 20 subjects in a Japanese family, the alleles at the MCT118 locus inherited according to the rules of Mendelian genetics. These data shows that MCT118 will be a valuable for paternity testing and for forensic identification of individuals in Japanese.

A 70

PATERNITY TESTING BY DNA ANALYSIS WITH THE USE OF VNTR MARKERS.

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

DNA analyses by VNTR markers were applied to three cases of paternity testing. Both in case of paternity and in case of paternity exclusion, RFLPs in VNTR loci were useful. In the third case, in which the alleged father and his first wife were deceased, his genotypes were determined from his second wife and four acknowledged children, and were compared to the paternal alleles of another child, the plaintiff of the case. The probabilities of paternity obtained from 7 red blood antigens, 5 red cell enzymes, 15 serum proteins, 3 loci of human leukocyte antigens and RFLPs including 4 VNTR markers were 0.586, 0.661, 0.997, 0.950 and 0.974, respectively, and the combined probability was 0.9999987. In DNA analyses, hyper-variable RFLPs in VNTR loci were useful, but diallelic polymorphisms were useless in these cases. The utility and problems in the application of VNTR markers to paternity testing were discussed.

A 71

Genetic polymorphism of gene conversion within the duplicated human α-globin loci.
Hitoshi NAKASHIMA, Asao FUJIYAMA, Takashi IMAMURA (Dept. of Hum Genet., National Institute of Genetics., Mishima)

The identification of individuals possesing one or three adult α-globin genes on a single chromosome, instead of the normal duplicated α-globin genes, provides strong genetic evidence for the intergenic recombination mechanisms, such as unequal crossover and gene conversion, in the evolution of human multigene families. Additional evidence that this tandem arrangement of homologous α-gene sequences promotes unequal recombination is the production of DNA deletions, which are indistinguishable from those found in the human α-thalassemia genes, upon propagation of the cloned α-globin gene DNA in Escherichia coli. Although the duplicated human α-globin genes encode identical polypeptides, previous studies have establishd that the α 1 and α 2 genes are not identical at the DNA level. This finding could be inferred by alignment of the restriction maps of the α-loci residing on the one-, two-, and three-gene chromosomes. We have identified 10 individuals heterozygous for a chromosome with the triplicated α-globin loci of the 645 Japanese subjects studied. The frequency of the triple α-loci was 0.008 in this population, while that of the single α-locus, i.e. α-thalassemia 2 gene, might be lower than 0.0008. We completely sequenced 2,132 bases of the third α-globin(α 3) gene region and identified that α 3 locus is constructed with α 1 and α 2 locus. Furthermore we analysed 10 triplicated α-globin genes about *Rsa* I RFLP linking to the α-globin gene, and identified these triplicated α-globin genes are not single origin.

A 72

PHYSICAL MAPPING OF CHARACTERISTIC JAPANESE MHC HAPLOTYPES

JIN Feng[1], Katsushi TOKUNAGA[2], Yoshihisa WATANABE[1], Keiichi OMOTO[1], Tohru NAOHARA[3], Takeo JUJI[2] ([1] Dept. Anthrop., Univ. Tokyo, Tokyo; [2] Blood Transfus. Serv., Tokyo Univ. Hosp., Tokyo; [3]Centr. Red Cross Blood Cent., Tokyo)

Long range physical mapping of HLA Class II and III regions in eight kinds of characteristic Japanese MHC haplotypes have been performed by pulsed-field gel electrophoresis(PFGE) and Southern hybridization with five rare cutting restriction endonucleases(*Mlu* I, *Not* I, *Nru* I, *Pvu* I, and *Sal* I) and various MHC cDNA probes. The results showed that there was no extensive difference of MHC genome organization among the haplotypes A11/24-Bw54-DR4, A11-Bw62-DR4, A26-Bw61-DR9, and A2-Bw46-DR9. However, deletions totaling about 100, 100, 120, and 190 kb were found in Class II region of the haplotypes A24-B7-DR1, A24-Bw52-DR2, Aw33-B44-DRw13, and A2-Bw46-DRw8, respectively. The haplotype A24-B7-DR1 possesses about 60 kb duplication in which 21-OHA and C4A are involved. The signal intensities of hybridization were nicely correlated to the gene copy number differences. These results reveal that (1) the structure of each characteristic MHC haplotype has been well conserved during human evolution; (2) the length of Class II region mostly depends on the DR allele; (3) PFGE-Southern analysis is useful for the estimation of gene copy numbers; (4) in relation to the proposed human genome project, it is of interest whether such major deletions or insertions exist in other chromosomal regions.

A 73

INTRASPECIFIC NUCLEOTIDE SEQUENCE DIFFERENCES IN THE MAJOR NONCODING REGION OF HUMAN MITOCHONDRIAL DNA.
Satoshi HORAI and Kenji HAYASAKA(Dept. Human Genet., National Institute of Genetics, Mishima)

Nucleotide sequences of the major noncoding region of human mitochondrial DNA (mtDNA) from 95 human placentas have been determined. These sequences include at least 482 base-pair (bp) long region encompassing most part of the D-loop forming region. Average nucleotide diversity among the sequences is estimated as 1.45%, which is three to four-fold higher than the corresponding value estimated from restriction-enzyme analysis of whole mtDNA genome. More than 97% of the base changes are transitions. The phylogenetic analysis indicates that diversity among the Negroids is much larger than that among the Caucasoids or the Mongoloids. A striking finding in the phylogenetic analysis is that the Mongoloids can be separated into two distinct groups. Divergence of part of the Mongoloids follows the earliest divergence of the part of the Negroids. Remainder of the Mongoloids subsequently diverged together with the Caucasoids. This observation confirmed our earlier study which clearly demonstrated existence of two distinct groups in the Japanese by the restriction enzyme analysis.

B 1

A Case with the distal long arm monosomy chromosome 1 due to de novo translocation.
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The patient,a female,was the first child of 40-year-old mother and 39-year-old father. She had been concieved as a result of artificial fertilization,utilizing a combination of ovulatory agents administration and the Pacol method and was delivered on the 2nd day after 41 weeks. Her birth weight was 2,500g ,and it was found at the same time that she suffered from hypoglycemia and hyponatremia. She also suffered patent ductus arteriosus and tricuspid regurgitation. Radiological examination revealed spina bifida,cephalic MRI,and absene of the corpus callosum. The clinical symptomes were brachycephaly, shortennd neck,frontal prominence,deformed auricles, oblique palpebral fissures,raised cheek bones, micrognathia,and subcutaneous edema. A karyotype revealed the attachment of an accesory structure to the end of the long arm of chromosome 1,and in addition,an NOR band was detected at its base,using the silver staining method. Analysis with a high resolution banding method showed a break at the region of 1q42.1->qter. However, the origin of the accessory structure translocation to the 1q terminal could not be identified. The karyotype of the parents was found to be normal.

B 2

13q3 monosomy 症候群の母子の軽症例と多発奇形を合併した重症例－－親子例の報告と症状の多様性についての考察．家島　厚[1]・頼田多恵子[2]（[1]鳥取県皆生小児療育センター，[2]鳥取大脳研小児科）．FAMILIAR MILD CASES AND A SEVERE CASE OF MONOSOMY 13q: Atsushi IESHIMA[1] and Taeko YORITA[2] ([1]Tottori Prefec. Kaike Rehabili. Cent. for Disabled children; [2]Div. Child Neur. Tottori Univ. Sch. of Med., Yonago)

13q3 monosomy は、既に 数多くの報告例があり、症候群として確立されている。今回、13q3 monosomy 症候群の非常に軽症な親子例を経験した。また、 脳奇形、外性器奇形などを合併した重症例も報告し、症状の多様性について考察する。

症例１：１才９ケ月女児．精神運動発達遅滞と先天性心疾患のため染色体検査を受け、13q32 monosomy と診断された。顔貌の特徴以外、13q3 monosomyの特徴はなかった。母親の染色体検査で、同様の核型と診断。軽度の知的な遅れと顔貌で両眼開離などの本症の特徴が見られた。母親が妊娠中のため羊水検査を行った所、本症と診断。剖検では、異常を認めなかった。症例２：８才男児．染色体は、13q32 monosomy。主要症状は、重度精神運動発達遅滞（10ケ月レベル）、脳奇形（全前脳胞症，小脳脳幹低形成）、虹彩欠損、脈絡膜欠損、Klippel-Feil奇形、男性仮性半陰陽（尿道下裂、小陰莖、潜伏睾丸）思春期早発症，好中球分葉核異常（２核まで）と特異顔貌（両眼開離、大きな切歯）である。切断部位が同じでも、症状が多様である理由として、欠失部位の相同染色体での劣性遺伝子の違いと考えている。

B 3

A CASE OF INTERSTITIAL DELETION OF THE LONG ARM OF CHROMOSOME 16. Masataka FUJIWARA, Makoto KAMADA (Dept. Pediatr., Hakodate Municipal Hospital, Hakodate) and Norihiko SHIMOYAMA (Dept. Pathol., Hakodate Municipal Hospital, Hakodate)

Partial deletion of the long arm of chromosome 16 is very rare; 12 cases have been published, but only one case, reported by Naritomi et al., has been known in Japan. Fryns et al. described the first case of them and proposed a new clinical entity "Fryns' syndrome". The propositus was a female baby born after 38 weeks' gestation. The pregnancy and delivery were uncomplicated. She weighted 2,678g at birth. Emesis after each feeding and frequent watery diarrhea began soon after birth. Severe dehydration and electlorite imbalance were followed by recurrent convulsions and cardiac arrests. She failed to thrive and appeared many minor anomalous, which is often observed in Fryns syndrome, for example, high forehead, large anterior fontanel, hypertelorism, upward slants of palpebrae, broad nasal bridge, narrow thorax and wide-set nipples. The father was 38-year-old and healthy. The mother was 29-year-old. Gravida I. Para I. She had been treated with anticonvulsants for Epilepsy since age 12, and had taken them during the pregnancy. They were not related and had no familial history of malformation. High resolution banding method revealed the Karyotype of the patient was 46,XX,del(16)(q22.1q22.3). This result indicate that the q22 is critical for this syndrome.

B 4

A CASE OF DOWN SYNDROME WITH MIRROR IMAGE DUPLICATION OF CHROMOSOME 21: 46,XY,-21,+dic(21)(pter→q22.3::q22.3→pter)

Satoshi ISHIKIRIYAMA (Div. Med. Genet., Chiba Children's Hospital, Chiba), Mizue IAI (Div. Neuro., Chiba Children's Hospital, Chiba), and Katsura ARAI (Dept. Pedodontics, Hokkaido Univ., Sapporo)

A 9-month-old boy had bilateral epicanthi, strabismus, depressed nasal root, macroglossia, bilateral tibial arches, and developmenal delay. Chromosome analysis with GTG-banding revealed one normal chromosome 21 and a tandem duplicated chromosome 21. Both of bands q22.2 remained on a duplicated chromosome 21. Thus the karyotype was 46,XY,-21,+dic(21)(pter→q22.3::q22.3→pter). The karyotypes of his parents were quite normal. Cytogenetical analysis by QFQ-banding indicated that parental origin of the dic(21) was paternal. Though we comfirmed positive stain at both ends of every dic(21) both by Ag-banding and by C-banding, we could find only one pair of dots at one of the ends of every dic(21) by Cd-banding. In this case only one of two centrmeres was active, and the other one inactivated. We have established Ebstein-Barr virus-transformed lymphoblastoid cell line of the boy. It may be possible to study the mechanism for inactivation of centromeres utilizing this cell line.

B 5

CELL DEVISION KINETICS OF THE RING CHROMOSOME 22: DOES "RING SYNDROME" EXIST?

Ryozo KASAI (Asahigawa Jidoin Child. Hosp., Okayama), Kouji NARAHARA, Yuji YOKOYAMA, Masae MURAKAMI, Hiroshi KIMOTO (Dept. Pediatr., Okayama Univ., Okayama) and Naoki KATAOKA (Dept., Pediatr., Kawasaki Med. Univ., Kurashiki)

In order to determine whether the "ring syndrome" exist or not, we examined cell devision kinetics of r(22) in 3 cases with an apparently telomeric fusion (breakpoints at p11.2 and q13.33). The gene dose effects for DIA1 and ARSA were normal. Clinically, psychomotor retardation was present in all the 3 cases, but growth failure and microcephaly was noted in only one case (case 1). Analysis of ring forms in the 72 hr lymphocyte cultures showed that frequencies of cells with monosomy 22, dic r(22) and other ring variants were 3.8 %, 1.0 % and 2.4 % in case 1, 5.5 %, 0.5 % and 0 % in case 2 and 3.6 %, 1.5 % and 2.0 % in case 3, respectively. The ratio of cells with monosomy 22 plus dic r(22) appeared to be constant in 3 rounds of cell cycle, indicating that the ratio was the most reliable index representing the *in vivo* instability of the ring chromosome. There was no obvious correlation between the clinical findings and the frequencies of cells with monosomy 22 plus dic r(22) in the cases with r(22). It was concluded that the difference in clinical manifestation of r(22) may be due to the varied extent of the submicroscopic deletion rather than to the ring instability.

B 6

A t(14;14) CLONE EXISTS ONLY IN A CD8+ SUBSET IN ATAXIA TELANGIECTASIA.
Keiko WAKUI, Toshiro NISHIDA (Dept. Clin. Lab., Saitama Child. Med. Ctr., Saitama), Tsutomu OHISHI (Div. Immunol., SCMC), and Yoshimitsu FUKUSHIMA (Div. Med.Genet., SCMC)

A 15-year-old girl with typical ataxia-telangiectasia (AT) had an AT clone with t(14;14) rearrangement involving approximately 90 % of the metaphases of phytohemagglutinin (PHA)-stimulated peripheral lymphocytes. Breakpoints of t(14;14) were 14q11 and 14q32. There was an extra band resembling to 14q32 between 14q32 and 14q11. We separated T-cells and B-cells by Ficoll-Paque density gradient centrifugation of mixture of pheripheral lymphocytes of the patient and sheep RBCs. A CD4+ subset was obtained by incubating a part of T-cells and anti-CD8 antibody with complement. PHA, interleukin 1 (IL1) and 2 (IL2) were added to T-cells or a CD4+ subset, and staphylococcus aureus Cowan I (SAC), IL1 and IL2 to B-cells. We harvested the cells after 3-day culture and analysed a karyotype by G-banding. A t(14;14) rearrangement was found in 90 % of metaphases of T-cells, but not in B-cells nor in a CD4+ subset. Thus, it is likely that a t(14;14) rearrangement was occured only in a CD8+ subset.

B 8

DELETION MAPPING OF PLASMINOGEN (PLG) AND α-L-FUCOSIDASE (FUCA2) IN A CASE WITH DEL(6q).
Kouji NARAHARA, Tsunenori MATSUBARA, Hiroshi NAMBA, Yuji YOKOYAMA, Hiroshi KIMOTO (Dept. Pediatr. Okayama Univ., Okayama) and Ryozo KASAI (Asahigawa Jidoin Hosp., Okayama)

We examined gene dosage effects for PLG and FUCA2 in a del(6q) patient with mental retardation, congenital hydrocephalus and craniofacial dysmorphisms. High resolution banding analysis showed the karyotype of 46,XY,del(6)(q2605::). The gene dose studies demonstrated single dosage effect for PLG but duplex effect for FUCA2, being consistent with the assignment of PLG to 6q2605-qter and exclusion of FUCA2 from this region.

The father was found to have the apparently same chromosome abnormality but to have a normal level of PLG. In situ hybridization study using the probe for PLG showed the accumulation of signals on the terminal portions of 1q, 6q, and 22q. Diligent cytogenetic study confirmed t(1;6)(q44;q2605) in the father. The usefullness of the in situ hybridization method in clarifing the subtle structural abnormality was stressed.

B 9

A SPORADIC CASE WITH ANIRIDIA AND WILMS' TUMOR ASSOCIATIN WITH A DE NOVO INVERTED INSERTION INV INS(10;11)(p13;p13 p15.1). Kiyoshi IMAIZUMI, Fumio TAKADA, Yoshikazu KUROKI. (Div. Med. Genet. Kanagawa Child. Med. Cent.).

A girl aged 2-year-6-month suffered from aniridia and Wilms' tumor. Neither malformations nor mental retardation were noticed. Cytogenetic analysis with GTG banding revealed abnormal 46,XX,inv ins(10;11)(p13;p13p15.1). There seemed to be no apparent chromosomal loss. Chromosome analysis of the parents were normal. Most of aniridia-Wilms' association predispose to associate with a chromosomal deletion, while a balanced translocation with breakpoint at 11p13 were reported in several isolated aniridia cases. The patient reported here suggested that an apparent balanced insertion might result in a loss of aniridia-Wilms' tumor genes by submicroscopic deletion or disruption of the genes at 11p13.

B 10

DNA DELETION STUDIES ON PRADER-WILLI, ANGELMAN, WILLIAMS, AND COHEN SYNDROMES. Jun-ichi HAMABE, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Yoshikazu KUROKI, Kiyoshi IMAIZUMI (Div. Med. Genet., Kanagawa Child. Med. Cen., Yokohama)

DNA deletion studies were performed in a total of 63 patients with various disorders in which the chromosome abnormality involving 15q11-12 region has been reported. Patients included 48 cases of Prader-Willi syndrome (PWS), 9 of Angelman syndrome (AS), 4 of Williams syndrome (WS) and 2 of Cohen syndrome (CS). Of 48 PWS patients, 37 have typical clinical features and 11 are atypical cases lacking several symptoms and having normal chromosomes 15. The probes used in this study were pML34, pTD3-21, p15NJ4, p15NJ65, and p15NJ118. With either or both of pML34 and pTD3-21, one-copy density was detected in 28 typical PWS patients and 3 AS patients, while no patients with CS and WS showed one-copy signal. With the microdissection/microcloning technique we established, we have obtained a total of 2×10^4 clones from 15q11.2 region and screened them to find fragments representing one-copy signal. Of 30 clones screened, p15NJ4 (a 500 bp fragment), and both p15NJ65 (550 bp) and p15NJ118 (400 bp) showed one-copy signal in 2 AS patients, and in a PWS patient who has normal karyotype, respectively. We are now searching DNA fragments that would show one-copy signal in an all-or-nothing manner among 48 PWS and/or 9 AS patients.

B 11

EFFECT OF SEQUENTIAL TREATMENT WITH FLUORODEOXYURIDINE AND EXCESS THYMIDINE ON FRAGILE X EXPRESSION. Kazushiro TSUJI, Kouji NARAHARA, Yuji YOKOYAMA, Kei HIRAMOTO, Hiroshi KIMOTO (Dept. Pediatr. Okayama Univ., Okayama) and Ryozo KASAI (Asahigawa Jidoin Hosp., Okayama)

The exact mechanism by which fragile X is produced is unclear. It has been known that various procedures which deplete the cellular pool of deoxythymidine and deoxycytidine induce the fragile X expression. We examined effects of a sequential treatment with fluorodeoxyuridine (FUdR) and excess thymidine on fragile X expression in 4 male patients with fragile X syndrome. Blood lymphocytes were cultured in medium RPMI 1640 supplemented with 10% dialyzed fetal calf serum for 96 hr. FUdR (10^{-6} M) and thymidine (5×10^{-4} M) were added 24 and 6 hr before harvest, respectively. The results showed that the frequency of fragile X expression was enhanced synergistically in 2 cases, but not in the remainders. The two types of response to the procedure may reflect the individual difference in DNA sequences of the putative gene for the fragile X syndrome. We believe that the procedure is more potent in producing the fragile X than with the usual method using FUdR.

B 12

Early-Replicating Fragile X Chromosome in Mentally Retarded, Carrier Women: A BrdU Antibody Study. Hirofumi OHASHI, Akira KUWANO, Yoshitsugu SUGIO, Masato TSUKAHARA, Tadashu KAJII (Dept. Pediat., Yamaguchi Univ. Sch. Med., Ube), Tadao ARINAMI (Dept. Hum. Genet., Univ. Tsukuba, Tsukuba)

Severe mental retardation in one third of the carrier women of the fragile X chromosome is assumed to be due to skewed X inactivation, an assumption repeatedly studied but never adequately substantiated. We thus studied the inactivation patterns of the fragile X chromosome in six carrier women, aged 14 to 37 years, and with an IQ range of 18 to 59. The fragile X was induced by adding 0.1 μM FUdR for the last 24 h of culturing lymphocytes in Eagle's MEM with 10% fetal calf serum. In order to minimize the fragile site-suppressing effect of BrdU used in the replication study, a BrdU antibody method was developed using 0.2 μg/ml BrdU and a commercial kit from Amersham. The results of early pulse and late continuous labeling were averaged. With this method, the rate of early replicating fragile X ranged from 23% to 73% in the six women studied.

B 13

ATKIN-FLAITZ SYNDROME: REPORT OF A FAMILY AND INTRAFAMILIAL VARIATION OF CLINICAL FEATURES. Ikuko KONDO(Dept. of Hum Ecol. & Genet., Univ. of the Ryukyus, Okinawa)

We report on a family with six moderately retarded individuals in two generations with the Atkin-Flaitz syndrome. Main clinical features of these patients are coarse facial appearance including prominent square forehead and supraorbital ridges, thick eye brows, broad nasal tip with anverted nostrils, thick lower lip and micrognathia. The hands are broad and short with short fingers. Short stature and mild macrocephaly are also observed in three and two individuals, respectively. However, three males aged at 20, 16 and 15 years have not macroorchisiam. Chromosomes were normal including fragile X analysis. X-ray findings of chest and hands were normal Based on clinical features of syndromes with coarse facial appearance, we diagnose this family having the Atkin-Flaitz syndrome. However, clinical features in families with this syndrome in the literature were slightly different. In addition, dysmorphic features in the family members are slightly different. Therefore, we propose that variability of clinical features of the Atkin-Flaitz syndrome, not only between families but within family members occure in this disease.

B 14

FAMILY STUDY ON THE FRAGILE X SYNDROME IN OKINAWA: ESTABLISHMENT of PHENOTYPE IN YOUNG MALES. Ikuko KONDO(Dept. of Hum. Ecol. & Genet., Univ. of the Ryukyus, Okinawa)

To establish clinical phenotypes of the fragile X(FraX) syndrome in pre-pubertal males, we have studied causes of mental retardation in 97 institutionalized patients aged from 5 to 26 years. After taking family history of mental retardation, patients were examined minor anomalies. Fifteen cases had Down syndrome and mental retardation resulted from problems at derivary and infections in 21 intimates. In 24 cases from 21 families, family history was positive. Fra(X) chromosome was detected in 2 to 15 % of cells from four boys aged 10, 14, 15 and 16 years. In addition, two boys aged 8 and 2 years were diagnosed as having Fra(X) chromosome following the family study. Based on these young boys with Fra(X) syndrome, we point out that square face with puffy cheek, high arched eyebrows, protruded pointed chin, big ears, and pucker up lips, puffy hands and high stepping gait may be characteristic in young boys with Fra(X) syndrome.

B 15

NEW HERITABLE RARE FRAGILE SITE fra(3)(p25) Hisako OCHI, Shaw WATANABE National Cancer Center Research Institute, TOKYO

A new heritable rare fragile site at band 3p25 was found in a 67-year-old male patient with pancreatic cancer. Peripheral lymphocytes were obtained from the proband before operation, and were cultured for detection of fragile sites. Chromosome analysis of the proband showed that chromosome 3 was broken at p25 in 17% of metaphases examined under the culture condition used for detecting distamycin A-inducible fragile sites, whereas no gap nor break was found in metaphases under the culture conditions to detect folate-sensitive fragile sites or BrdU-requiring ones. The lymphocytes from his two sons were examined under the condition to detect distamycin A-inducible fragile sites, and revealed to have gaps or breaks at the same 3p25 in 18% and 24% of metaphases respectively. Thus, this fragile 3p25 band is considered to be a new rare fragile site induced by distamycin A. Moreover, the carriers of this heritable rare fragile site possibly have a predisposition to develop cancer, because raf-1 oncogene is located on 3p25 and brother of the proband died of pancreatic cancer.

B 16

POPULATION CYTOGENETICS ON HERITABLE FRAGILE SITES IN JAPANESE POPULATIONS. Tada-aki HORI, Ei-ichi TAKAHASHI (Div.Genet., Natl.Inst., Radiol.Sci.,Chiba), Kunikazu KISHI (Sch.Health Sci.,Kyorin Univ.,Tokyo) Akira HOMMA, Riichi IMAMURA(Sec.Psychiat.,Tokyo Metropl.Inst.Gerontol., Tokyo), Naohiko SEKI (Sch. Med., Chiba Univ., Chiba) and Motoi MURATA (Dept. Epidemiol., Chiba Cancer Center, Chiba)

We have conducted population survey for heritable rare fragile sites in healthy subjects (1022), cancer patients (693), institutionalized mentally retarded patients (364) and azoospermia patients (35). The following 13 rare fragile sites were detected: Folate-sensitive, 2q11.2 2q13, 11q13.3, 11q23.3, 12q24.1, 17p12, Xq27.3; Distamycin A-inducible, 8q24.1, 11p15.1, 16p12.1, 16q22, 17p12; BrdU-requiring, 10q25.2. The overall frequency of fragile site carriers was 6.3% (131/2114) and the majority (84.0%) of them were distamycin A-inducible fragile sites. Three fragile sites at 8q24.1, 11p15.1 and 16p12.1 appear to be endemic to the Japanese population. Although both fra(16)(q22) and fra(17)(p12) and nonfragile allelomorphs seem to exist as chromosomal polymorphisms, significantly higher incidences of fragile site carriers were noted in preleukemic disorders, gynecological benign tumor, lung cancer, mentally retarded patients with chromosome abnormalities, and azoospermia patients. This suggests that distamycin A-inducible fragile sites are not completely harmless chromosomal variants, but may be sites involved in chromosome instability of human genome.

B 17

REPAIRING PATTERN OF APHIDICOLIN-INDUCED COMMON FRAGILE SITES: T LYMPHOCYTES.

Ichiro MURANO, Akira KUWANO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med. Ube)

Aphidicolin added 24 h before harvest at a concentration of 0.2μM induces frequent common fragile sites in peripheral blood lymphocytes cultures. In order to study the repairing process of common fragile sites, aphidicolin was removed 2, 4, and 6 hours before harvest in lymphocyte cultures from two voluteers, a 29-year-old man and a 35-year-old woman. Mean total breaks in 50 metaphases each were 217.5 in control culture, 52.8 in 2 h releasing culture, 17 in 4 h, 7.3 in 6 h. The distribution and frequency of fragile sites were studied in the control and 4 h releasing cultures. The sites at 16q23 and Xp22.2 were almost completely repaired after a 4 h release, while 11.4-46% of breaks of control cultures remained under the same condition un-repaired in those at 3q14, 6q26 and 9q13.

Fragments, dicentrics and triradials were increased with increasing release time, whereas chromatid interchanges were decreased.

B 18

Repairing patterns aphidicolin-induced common fragile sites: B lymphoblastoid cells.

Yukihisa MATSUDA, Akira KUWANO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Aphidicolin 0.2μM, added 26 h prior to harvest of EB virus established B lymphoblastoid cells, induces common fragile sites. Its repairing patterns were studied by washing aphidicolin 4 h prior to harvest. B lymphoblastoid cells from 5 volunteers were studied. The repairing rate of total fragile sites was 77.5%, of individuals fragile sites, it ranged from 47.5% to 83.1% for 7q11.2, 4q23 and 3p14, while it was 100% for 16q23. As for structual abnormalies, 4 h release resulted in an increase of fragments, dicentrics and triradials, whereas chromatid interchanges were decreased.

B 19

THE RFLP ANALYSES OF THE RISK FACTORS FOR CORONARY ARTERY DISEASE AND HYPERTENSION IN JAPANESE POPULATION.

Ryuji KAWAGUCHI, Kazuo TAKEMURA, Ryoji MORITA, Keiko HASEGAWA, Takanobu SAIGOH, Kazumasa HIKIJI, Yutaka TSUKADA, Masayoshi TAKAHASHI (GRL, SRL), Michio ARAKAWA, Chisato HIRAKAWA (2nd Int. Med., Gifu Univ., Gifu), Masako KUSUMOTO, Shinichi KIMATA (Heart Inst. Tokyo Women's College, Tokyo)

Restriction fragment length polymorphisms (RFLP) were studied in 522 Japanese population having 164 coronary artery diseases, 143 hypertension, 65 diabetes mellitus, 32 hyperlipidemia, 164 normal control and so on, using 14 polymorphic DNA probes (Apo AI, Apo CIII, 3'Apo AIV, 5'Apo B, 3'Apo B (1.6 & 1.5 kb), Phospholipase A2, basic FGF, 5'Renin, ANP, Apo CI, beta Na-K ATPase and insulin) and 10 restriction enzymes. The frequency distribution for markers, Dra I/Apo C I and Taq I/phospholipase A2 was completely agreed with that for Caucasians, and that for probes Taq I/Apo A IV and Pvu II/5'Apo B was slightly different ($p=0.15$ and 0.55, respectively). However, it was quite different for the other 20 markers ($p<0.01$). A correlation was found between a marker Eco RI/3' Apo B and the patients with stenosis in age 12 to 50 determined by coronary arteriography ($N=89$, $p=0.02$). The higher correlation was found between Taq I/Renin and essential hypertension ($N=221$, $p=0.008$), as well as Hind III/beta Na-K ATPase and hyperlipidemia ($N=114$, $p=0.007$).

B 20

ASSOCIATION OF APOE4 AND HYPERCHOLESTEROLEMIA IN SCHOOL CHILDREN.

Hisako YANAGI[1], Yae SHIMAKURA[2], Yuka WATANABE[1], Yasuko YAMANOUCHI[1] and Hideo HAMAGUCHI[1] ([1]Dept. Hum. Genet., Inst. Basic Med. Sci., [2]Inst. Clin. Med., Univ. of Tsukuba, Tsukuba)

Genetic factors responsible for the cholesterol levels in adults have been comming clear. One of them is APOE phenotypes. We reported the association of APOE4 and hypercholesterolemia in Japanese adults, previously. To investigate whether APOE4 is associated with hypercholesterolemia in children, we studied the APOE phenotypes of 51 school age children with primary hypercholesterolemia and of 51 age, sex and obesity index matched controls with normocholesterolemia by two-demensional gel electrophores. APOE4 was present in twenty-one of 51 hypercholesterolemic children (41.2%), and in nine of 51 control subjects (17.6%). The diffirence was significant ($p<0.01$). In addition, allele frequency of APOE4 was significantly higher in the hypercholesterolemic children ($\varepsilon4=0.235$) than in the control subjects ($\varepsilon4=0.088$) ($P<0.01$). This finding indicates that APOE4 is associated with hypercholesterolemia in children.

B 21

GENETIC POLYMORPHISM OF APOLIPOPROTEIN E AND HYPERCHOLESTEROLEMIA IN JAPANESE : V. APO E4 AND SERUM APOLIPOPROTEIN B LEVELS. Yasuko YAMANOUCHI[1], Shigeru TSUCHIYA[2], Yuki SAKAI[1], Hisako YANAGI[1], Ryunosuke MIYAZAKI[3] and Hideo HAMAGUCHI[1] ([1]Dept. Hum. Genet., Inst. Basic Med. Sci., [2]Inst. Comm. Med., Univ. Tsukuba, Tsukuba; Dep. Med., [3]Kudanzaka Hospital, Tokyo).

Apolipoprotein E (Apo E) phenotypes were determined in 808 apparently healthy unrelated Japanese adult males by two-dimensional gel electrophoresis (2-DE). These males visited a health care center in Tokyo for their annual health examinations. Gene frequencies were ε3=0.839, ε4=0.102, ε2=0.043, ε5=0.004, and ε7=0.008. Among 808 males, 402 were normal in all examinations. The gene frequencies in 402 normal males were almost identical with those in 808 subjects. The mean (±SD) serum apolipoprotein B (Apo B) level in fasting blood was 75.2±15.5 mg/dl in 194 normal males with apo E3/3 and 82.3±20.9 mg/dl in 49 normal males with apo E3/4. The difference was significant ($p<0.05$). Mean serum cholesterol levels and frequencies of hypercholesterolemia (cholesterol level ≧ 240mg/dl) were 189.9 ±30.6mg/dl and 6.7%, respectively, in 285 normal males with apo E3/3 , and 196.7±31.7 mg/dl and 12.3%, respectively, in 65 normal males with apo E3/4. The data suggest that ε4 raises serum Apo B levels compared with ε3 in Japanese.

B 22

ASSOCIATION OF DNA POLYMORPHISMS AT THE LOCUS FOR CHOLESTERYL ESTER TRANSFER PROTEIN(CETP) WITH HDL CHOLESTEROL AND APO AI LEVELS. Ikuko KONDO(Dept. Hum. Ecol. & Genet., Univ. of Ryukyus, Okinawa) and Kåre Berg(Inst. Med. Genet., Univ. of Oslo, Oslo)

Cholesteryl ester transfer protein(CETP) plays an important role in reverse cholesterol transport and anomalies of this protein may be involved in susceptability or resistance to atherosclerotic diseases. We have studied 146 unrelated Norwegian for DNA polymorphisms at the CETP locus detectable with the restriction enzyme Taq I. Highly significant differences in Apo AI concentration were found between the homozygotes with different alleles at Taq I polymorphic region and there was a dosage effect on Apo AI level of the gene. In addition, there was the most significant difference of Apo AI level between the two catergories of homozygotes in non-smokers and no significant difference in mean Apo AI level was found between genotypes in smokers. Mean HDL cholesterol level was also significantly lower in homozygotes with the common type of allele at the CETP locus than in those with the rare allele. We conclude that the DNA polymorphism at the CETP locus is likely to be related to biological function of Apo AI and HDL cholesterol metabolisms.

B 23

THE SUSCEPTIBILITY TO PSORIATIC ARTHRITIS AND HLA.
Masahiko MUTO, Chidori ASAGAMI (Dept. Dermatol., Yamaguchi Univ., Ube), and Tomokazu SUZUKI (Dept. Clin. Genet., Kyushu Univ., Beppu)

Psoriatic arthritis (PA) is considered a genetic disease. However the mode of inheritance of the susceptibility to PA has not been clear. In order to elucidate genetic involvements for the development of PA, we gathered 19 unrelated PA patients, according to a criteria proposed by Moll and Wright. Association study showed that a major histocompatibility antigen HLA-A2 was significantly increased in frequency. In addition, we studied the mode of inheritance of the HLA-A2-linked gene controlling susceptibility to PA, by using the method proposed by Thomson and Bodmer. Out of the 19, only 3 lacked the antigen HLA-A2, 13 were confirmed heterozygotes and 3 were suspected homozygotes for A2. The data were compatible with a dominant mode of inheritance ($X_1^2 = 0$). Thus, the susceptibility gene in linkage disequilibrium with HLA-A2 seems to be inherited in a dominant fashion.

B 24

CORRELATION OF MHC WITH RHEUMATOID ARTHRITIS (1):THE HIGH RISK HAPLOTYPES AND CLINICAL ASPECTS.
Fujio TAKEUCHI, Keiichiro NAKANO, Kunio MATSUTA, Eri KOSUGE, Terumasa MIYAMOTO (Dept. Med. and Physc. Ther., Univ. Tokyo, Tokyo), Katsushi TOKUNAGA, Kazumasa MATSUKI, Takeo JUJI (Dept. Blood Transf.), Yoshihisa WATANABE, (Dept. Anthropol.) and Hiroo MAEDA (Dept. Blood Transf. Saitama Med. School, Saitama)

The genetic background was investigated in RA patients. The frequency of RA in 4523 family members (within 3-degrees) from 180 RA patients was 1.97% and was higher than that in 1536 family members from 69 control subjects (0.46%). Population study and familial study indicated the existence of 2 different putative susceptibility haplotypes :HLA-Bw54/59-C2C-BFS-C4A3-C4B5-DR4.1-DQw4 and C2C-BFS-C4AQ0-C4B1/2. C4AQ0 and C4B5 might play roles in the pathogenesis of RA independently. The patients with C4AQ0, C4B5 or HLA DR4.1 showed higher familial incidence, later onset of the disease or faster progression than those without these genetic markers but these were not significant. There were not differences for rheumatoid factor, rheumatoid nodule and extra-articular signs between those two groups. The discriminative analysis was carried out tentatively for 51 patients in the method of Hayashi, using C4AQ0 and C4B5 for outside variables, and age of onset, position of the first symptom, position of the first deformity, sense of cool, duration till deformity and familial history for items. Patients were classtered in 4 groups.

B 25

CORRELATION OF MHC WITH RHEUMATOID ARTHRITIS: (2) PUTATIVE AMINO ACID SEQUENCE OF HLA-DR THAT CONTRIBUTES TO RA SUSCEPTIBILITY. Yoshihisa WATANABE[1], Katsushi TOKUNAGA[2], Kazumasa MATSUKI[2], Hiroo MAEDA[3], Keiichi OMOTO[1], Fujio TAKEUCHI[4], Kunio MATSUTA[4], and Takeo JUJI[2]. ([1]Dept. Anthrop., [2]Blood Transfusion Serv., [4]Dept. Int. Med. and Phy. Ther., Univ. of Tokyo, [3]Blood Transfusion Serv., Saitama Med. Sch.)

To clarify the determinant of susceptibility to RA, a polymorphic segment of the HLA-DRB gene was amplified *in vitro* by PCR and analyzed with oligonucleotide probes specific for the HLA-DR4 DNA sequences. Comparison of 90 RA patients and 47 controls revealed that a particular sequence encoding amino acids Gln^{70}-Arg^{71}-Arg^{72}-Ala^{73}-Ala^{74} showed a strong association with RA ($P<0.00001$, relative risk 7.2). This amino acid sequence is shared among three RA-associated specificities, DR4/Dw14 ,DR4/Dw15, and DR1. DR4/Dw4 which is common in Caucasian RA patients has a strikingly similar amino acid sequence Gln^{70}-Lys^{71}-Arg^{72}-Ala^{73}-Ala^{74} in terms of polarity and charge profiles. Other RA non-associated sequences differ from this sequence by at least one amino acid substitution that alters the net charge. The composition of amino acid residues at the position 70 to 74 may play a crucial role in the pathogenesis of RA. Of great interest is that this sequence also occurs in the gp110 glycoprotein of EB virus (Roudier *et al.*, 1989). The virus would be a triggering factor of RA.

B 26

HLA AND IMMUNOREACTIVITY IN TAKAYASU ARTERITIS Fujio NUMANO, Ryutaro MORIWAKI, Kenji KASUYA, Junko MITANI, Michiyoshi YAJIMA, Koichi SUZUKI*, Hideo MATSUMOTO*, Yasuharu NISHIMURA**Takehiko SASAZUKI**Tokyo Med. & Dent. Univ., Tokyo, *Osaka Med. Col., Osaka, **Kyusyu Univ., Fukuoka.

Recent studies on HLA in Takayasu arteritis revealed a statistically significant high frequency of extended haplotype of HLA A24-Bw52-C4A2-$C4BQ_0$-Dw12 in these patients(Numano,F.et al:Ex.Clin. Immunogenet.6:236 1989). Takayasu arteritis is known its immunoreactivity. We measured antinclear antibody(ANA), CRP, ASO, RA, IgA, IgG, IGM, C_3, $C_4$and CH_{50} in 42 patients of Takayasu arteritis, compared with 50 controls, to study their association with this extended haplotype. There were no statistically significant difference between both groups in the levels of ANA, RA and ASO. However the frequency of positive CRP, was high in diseased group(χ:7.8 $\gamma\gamma$0.34 $p<0.01$) and the levels of IgM($p<0.05$), IgA($p<0.01$) and C_3($p<0.01$) were confirmed to be significantly high in Takayasu arteritis, as compared with those in controls. These high levels were striking in 21 patients who carries Bw52(Bw52(+)). There was no difference in CH_{50} between patients and controls. However Bw52 (+) group exhibited a significantly high level of CH_{50}($p<0.01$) as compared with that of patients with Bw52(-), which may suggest the participation of hereditary factor disequilibrium with this extended haplotype to the morbid condition of Takayasu arteritis.

B 27

ANALYSIS OF GENES CONTROLLING RESISTANCE TO INSULIN DEPENDENT DIABETES MELLITUS (IDDM) IN THE JAPANESE. Yoshinori FUKUI, Takeshi INAMITSU, Akinori KIMURA, Yasuharu NISHIMURA,Takehiko SASAZUKI (Dept.Genet., Med.Inst.Bioreg.Kyushu Univ., Fukuoka)

To investigate HLA-linked genes controlling the susceptibility and resistance to IDDM, HLA-DQ alleles of 45 Japanese patients with IDDM were analyzed, using sequence specific oligonucleotide (SSO). DQA1*0301 and DQB1*04 were positively associated (R.R=6.6, Pc<0.05 and R.R=4.7 Pc<0.01) and DQA1*0103 and DQB1*0104 were negatively associated(R.R=0.2, Pc<0.01) with IDDM. DQA1*0103 and DQB1*0104 were in strong linkage disequilibrium to encode for DQw6 molecule. Thus, in a Japanese populatiion, the DQw6 molecule seems to control the resistance to IDDM. To determine whether or not the DQw6 molecule itself can protect against glycosuria and insulitis in NOD mice, these animals were mated with HLA-DQw6 transgenic-C57BL/6 mice (DQw6-B6)and the F1 progeny expressing the DQw6 molecule were backcrossed with NOD mice. Eighty-five female backcross progenies were classified into four groups, according to the MHC classll phenotype; I-A^{NOD}/I-A^{NOD} DQw6(-), I-A^{NOD}/I-A^{NOD} DQw6(+), I-A^{NOD}/I-A^{b} DQw6(-) and I-A^{NOD}/I-A^{b} DQw6(+). The glycosuria was checked weekly after age 15 weeks and the histological examination of pancreas was performed in the 30 weeks old mice or after the development of glycosuria. The DQw6 molecule seemed to delay the onset of glycosuria but did not protect against glycosuria and insulitis in the NOD mice despite a functional expression.

B 28

ANALYSIS OF HLA-DQB1 SEQUENCES BY PCR-RFLP: APPLICATION TO IDDM. Hiroshi IKEGAMI, Yoshihiro YAMAMOTO, Eiji YAMATO, Tepyon CHA, Hiroko YONEDA, Yoshihiko KAWAGUCHI, Yasuhiro TAHARA, Toshio OGIHARA (Dept. Geriatric Med., Osaka Univ., Osaka), Yoshihiko NOMA and Kenji SHIMA (Dept. Lab. Med., Tokushima Univ., Tokushima)

In order to further localize the HLA-linked diabetogenic gene, we have analyzed genomic sequences of DQB1 gene in Japanese patients with IDDM. With two oligonucleotide primers, 207 bp segment of DQB1 gene was amplified by the polymerase chain reaction (PCR), and amplified sequences were analyzed with allele specific restriction enzymes. By using three restriction enzymes, all but one alleles of DQB1 gene could be identified. We have applied this PCR-RFLP method to DNA analysis of HLA-DQB1 gene in 26 Japanese patients with IDDM. In contrast to Caucasian patients with IDDM, most (>70%) Japanese patients with IDDM had at least one aspartic acid at position 57 of DQ-beta chain. These data suggest that aspartic acid at position 57 of DQ-beta chain dose not protect Japanese from IDDM, and that other part of DQB1, DQA1, and/or DRB sequences may also affect disease susceptibility in Japanese IDDM.

B 29

ANALYSIS OF HLA CLASS II GENES IN NARCOLEPSY. Katsushi TOKUNAGA, Shoji KUWATA, Kazumasa MATSUKI, Takeo JUJI (Blood Transfus. Serv., Tokyo Univ. Hosp.), JIN Feng (Dept. Anthrop., Tokyo Univ.), Hidetoshi INOKO, Kimiyoshi TSUJI (Dept. Transplant., Tokai Univ., Kanagawa), Tsukasa SASAKI, and Yutaka HONDA(Seiwa Hosp., Tokyo)

Since our first report on the complete association of HLA-DR2(Dw2) and DQw1 with Narcolepsy, we have tested more than 250 patients. Present study focused on the possible major genomic arrangement specific to Narcolepsy. The patients and healthy controls with DR2/Dw2 or DR2/Dw12 was compared using rare-cutting restriction endonucleases (Pvu I, Not I, and Sal I) and pulsed-field gel electrophoresis. So far no Narcolepsy-specific extensive deletion or insertion has been observed. However, DR2/Dw2 was suggested to be some 10kb shorter than DR2/Dw12 in the DR-DQ region.

Also we established a convenient detection method for DR2/Dw2. DRB 2nd exon was amplified by PCR from only 100ul of peripheral blood. Hybridization with a Dw2-specific digoxigenin-conjugated oligonucleotide probe followed by enzyme immunoassay enabled a quick, safe and reliable typing of the specificity. This method will be useful in diagnosis and prevention of the disease.

B 30

Differences in the regulation of HLA class II genes.

Akinori KIMURA and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

There are four HLA class II molecules encoded for by the HLA class II multigene family which consists of α and β genes for the HLA-DR, DRw, DQ, and DP. HLA -DR genes serve as immuneresponse gene, while HLA-DQ gene may function as immunesuppression gene. To delineate the molecular basis for the functional differences between HLA-DR and DQ, we investigated the expression of the HLA class II genes, especially on the regulation by cytokines. In a glioblastoma cell line, T98G, TNFα induced HLA-DQ but not DR in combination with IFNγ. In contrast, IL6 reduced HLA-DQ but not DR. The induction of HLA-DQ by IFNγ + TNFα was mainly governed by the regulation of HLA-DQ α gene expression. By using CAT assay and gelshift assay, we have identified a nuclear factor which was induced by IFNγ + TNFα treatment and interacted specifically with DQα promoter. This factor was suggested to be similar to but distinct from NFκB. These data suggest that the DQα gene is under the quite different regulation from the other class II genes and may therefore be relevant to different functions in the regulation of immune system.

B 31

HLA-LINKED GENETIC CONTROL OF IMMUNE RESPONSE TO HBS ANTIGEN IN MAN. Ken HATAE, Hiroshi WATANABE, Ryoko OKUBO, Kuniyoshi TSUCHIYA, Nobuhiro KAMIKAWAJI, Akinori KIMURA, Yasuharu NISHIMURA, Takehiko SASAZUKI(Dept. of genetics,Med. Inst. of Bio-reg.,Kyushu Univ.) and Henry A. ERLICH (Cetus Corp. USA)

We have investigated the association between immune response to HBs vaccine and HLA alleles. The frequencies of serologically determined HLA-Bw54, Cw1, DR4, DRw53, DQw4 were increased and those of HLA-DR1, DQw1 were decreased in low responder group. DQA, DQB and DPB alleles were determined by testing the hybridization between genomic DNA amplified by PCR and sequence specific synthetic oligonucleotide probes. The significant increase of DQA1*0301, DQB1*0401 and significant decrease of DQA1*0101, DQA1*0103, and DQB1*0101 were observed in low responder group. These HLA alleles associated with immune responsiveness to HBs antigen are in strong linkage disequilibrium. Thus HLA-Bw54-Cw1-DR4-DRw53-DQw4 (DQA1*0301, DQB1*0401) is associated with low reoponsiveness to HBs antigen and HLA-B7-Cw7-DR1-DQw5 (DQA1*0101, DQB1*0101) is associated with high responsiveness. Proliferative response of peripheral blood lymphocytes and T cell lines to HBs antigen was completely inhibited by monoclonal anti-HLA-DR antibody in vitro. T cell lines responded to HBs antigen in the presence of mouse L cells transfected with HLA-DR genes. These observations clearly indicate that the majority of T cells recognize HBs antigen in the context of HLA-DR molecules. The mechanism of strong association between HLA and immune response to HBs antigen will be discussed.

B 32

ANALYSIS OF T CELL REPERTOIRE RECOGNIZING DQW6 IN THE HLA-DQW6 TRANSGENIC C57BL/6 MOUSE. Takeshi INAMITSU, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Genet.,Med. Inst. Bio-regul. Kyushu Univ.)

We have reported a functional expression of HLA-DQw6 genes in the C57BL/6 mouse (B6) as follows. 1) The HLA-DQw6 transgenic B6 mouse (DQw6-B6) did not produce antibodies directed against DQw6 molecule indicating the acquisition of tolerance to DQw6 2) B6 was low responder strain to the streptococcal cell wall antigen (SCW), whereas DQw6-B6 acquired a responsiveness to SCW. In this study, we have investigated the T cell repertoire recognizing specifically DQw6 molecule to elucidate the mechanisms for the changes of immune responsiveness in DQw6-B6. Lymphnode cells primed in vitro with irradiated spleen cells of DQw6-B6 exhibited a strong secondary mixed lymplogyte reaction (MLR) to DQw6 molecule expressed on DQw6-B6 spleen cell or DQw6 positive human monocytoes and these MLR were inhibited by anti DQ monoclonal antibody. On the other hand DQw6-B6 lymphnode cells primed in vitro with irradiated DQw6-B6 spleen cells did not show any MLR directed against DQw6 molecule. B6 T lymphocytes primed with DQw6 expressed V_β5, 6, 8 and/or V_α3.2. But there were no significant differences in the proportion of thymocytes or peripheral T cells expressing V_β3, 5, 6, 8, 9, 11 or V_α3.2 between B6 and DQw6-B6. A SCW specific T cell line restricted by DQw6 was established from DQw6-B6 and this T cell line recognized SCW in the context of DQw6 expressed on L cell transfected with DQw6 genes or DQw6 positive human monocytes. These responses were completely inhibited by anti DQ monoclonal antibody. Therefore the tolerance to DQw6 was acquired at T cell level and the DQw6 restricted antigen specific T cells were generated in DQw6-B6.

B 33

DISTINCT ROLE OF HLA-DR AND DQ MOLECULES IN IMMUNE RESPONSE

Nobuhiro KAMIKAWAJI, Hideyuki YOSHIZUMI, Ken HATAE, Akinori KIMURA, Yasuharu NISHIMURA, Takehiko SASAZUKI (Med. Inst. Bioreg. Kyushu Univ.)

We have elucidated the HLA-linked dominant immune suppression (Is) genes which controlled low immune responsiveness to the particular antigens through the induction of CD8+ suppressor T cells. Is gene to SCW is in strong positive or negative linkage disequiribrium with HLA-Dw12 or Dw15 haplotypes respectively. To investigate the function of the Is gene to SCW, IL-2 dependent T cell lines specific to SCW were established from Dw12 and/or Dw15 positive donors. Restriction molecules of T cells were identified by utilizing L cell transfectants expressing DQw6, DR2AB1 or DR2AB5 genes of Dw12 haplotype or DQw4, DR4 or DRw53 genes of Dw15 haplotype. Tcells restricted by DQw6 or DR2 were observed in low responders with Dw12,whereas only DR4 or DRw53 restricted T cells were observed in donors with Dw15 which is a high responder haplotype. Thus DQw4 restricted T cells were never obsereved in Dw15 positive donors including Dw12/Dw15 heterozyous low responders. Furthermore DQw6 restricted CD4+ T cells supported the growth of CD8+ T cells with suppressive activity, whereas DR4 restricted T cells did not. These observations suggested that DQ restricted CD4+ T cells may control low immune responsiveness through the activation of CD8+ suppressor T cells and that Is gene may be identical to HLA-DQ gene.

B 34

EVIDENCES FOR A POSSIBLE DISSOCIATION OF LIGANDS FOR T CELL RECEPTOR AND CD4 MOLECULE IN HLA-DQ RESTRICTED T CELLS.

Yasuharu NISHIMURA, Tooru SUDO, Takeshi INAMITSU, Nobuhiro KAMIKAWAJI and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

The specificity and activation of T cell is determined by the recognition of antigen in the context of HLA-class II molecules by T cell receptor(TCR) in man. This interaction between TCR and HLA-class II molecules is strengthned by the binding of CD4 molecule to the framework structure of HLA-class II molecule. The dissociation of ligans for TCR and CD4 molecule was suggested by the following observations in DQ restricted T cells. 1) Either anti-human CD4 or anti- HLA-DR monoclonal antibody inhibited the proliferative response of DQ restricted T cell line in human. 2) Either anti-mouse CD4 or anti-I-A monoclonal antibody inhibited the immune response of DQ restricted T cell line or T cell hybridoma in the HLA-DQ transgenic mouse. Because the expression of DR or I-A molecules is much higher than that of DQ molecule on antigen presenting cells, it is suggested that TCR of DQ restricted T cell recognizes antigen in the context of DQ molecule and that CD4 molecule binds to DR or I-A molecules expressed abondantly. On the other hand, anti-HLA-DQ monoclonal antibody did not inhibit the immune response of HLA-DR restricted human T cell line and I-A restricted T cell line or T cell hybridoma established from the HLA-DQ transgenic mouse. Therefore, the signal(s) for the activation of DQ restricted T cells may be different quantitatively or qualitatively from that of DR or I-A restricted T cells.

B 35

INVESTIGATION OF PHENYLKETONURIA HAPLOTYPE 2 AND 3 MUTATIONS AMONG SIXTY CHINESE PATIENTS Yoichi MATSUBARA[1], Hitoshi MIKAMI[1], Kuniaki NARISAWA[1], Keiya TADA[2], and Gui-Zhang DONG[3] ([1]Dept. of Biochemical Genetics and [2]Dept. of Pediatrics, Tohoku University School of Medicine, Sendai; [3]Dept. of Biochemical Genetics, The 3rd Hospital Attached to China Medical University, China)

Phenylketonuria (PKU) is an autosomal recessive disorder caused by mutations in the phenylalanine hydroxylase gene. Two types of mutations account for 60% of PKU mutations in the Northern European population and they are tightly linked to RFLP haplotypes 2 and 3 , respectively.

We have investigated sixty chinese patients for these mutations. Genomic DNA was extracted from dried blood spots on Guthrie cards and subjected to modified polymerase chain reaction (PCR). Amplified DNA was either hybridized with allele specific oligonucleotide (ASO) probes or analyzed by restriction enzyme digestion. None of the mutations were observed in sixty patients, indicating genetic heterogeneity between the two populations. The modified PCR method devised in our laboratory was found to be simpler and more rapid than the conventional ASO hybridization procedure.

B 36

ホモシスチン尿症の３例：大和田操[1]・北川照男[1]・藤田良二郎[2]・小板橋靖[2]
([1]日大・医・小児科、[2]聖マリアンナ医大・小児科). STUDY ON THREE CASES WITH HOMOCYSTINURIA : Misao OWADA[1], Teruo KITAGAWA[1], Ryojiro FUJITA[2] and Yasushi KOITABASHI[2] ([1]Dept. Pediatrics, Nihon Univ. Sch. of Med., Tokyo ; [2]Dept. Pediatrics, St. Marianna Univ. Sch. of Med., Kawasaki)

シスタチオニン合成酵素の遺伝的な障害に起因するホモシスチン尿症は、欧米では比較的頻度の高いアミノ酸代謝異常症であるが、我国では稀であり、その経過についても不明な点が多い。 そこで、我々の経験している本症の３例の臨床的、生化学的な経過を、これまでに報告されている30例の経過と比較した。 自験例３例中２例は水晶体脱臼、骨格異常、知能障害などの症状を呈して診断された年長例で、残る１例はマス・スクリーニングで診断された症例である。 B_6反応型の１例にはB_6大量投与を、B_6不応型には低メチオニン食治療を行ったが、診断時に認められたホモシスチン尿、高メチオニン血症、血小板機能異常は、治療によって改善した。 しかし、長期予後は必ずしも良くなく、１例では脳血栓を合併した。 B_6不応型が多い我国では、本症の治療に注意が必要である。

B 37

Characterization of mutations in two unrelated patients with ornithine transcarbamylase deficiency : Toshinobu MATSUURA[1,2], Akira HATA[1,2], Chiaki SETOYAMA[1], Kazunori SHIMADA[1], Takeshi SAKIYAMA[3], Toshinari NISHIKUBO[4], Teruo KITAGAWA[3], Kazuhiko OYANAGI[5] and Ichiro MATSUDA[2] ([1]Department of Biochemistry and [2]Department of Pediatrics, Kumamoto University Medical School, Kumamoto. [3]Department of Pediatrics, Nihon University School of Medicine, Tokyo. [4]Department of Neonatology, Shizuoka Children's Hospital, Shizuoka. [5]Department of Pediatrics, Sapporo Medical College, Sapporo.)

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 usually results in neonatal hyperammonemia, coma, and death in early childhood. In this report, we examined genomic DNAs derived from two unrelated patients with OTC deficiency. We found that one TaqI site in exon 5 of OTC gene is missing in a male patient who died three days after his birth. Subsequently, we cloned and sequenced one of his PCR amplified exon 5 DNAs and found that a C-to-T substitution is present in the TaqI site. This mutation changes the codon 141 for arginine to a stop codon. We demonstrated that characterization of the mutation is useful for prenatal diagnosis of OTC deficiency in his family. We also analyzed genomic DNA derived from a female patient with mild OTC deficiency by similar experiments, and found that a G-to-C substitution is also present in exon 5 of OTC gene. This mutation changes the codon 148 for leucine to phenylalanine. However, we have not yet proved that this mutation is directly related to OTC deficiency in this patient.

B 38

アルギニン血症の遺伝子解析. 原口洋吾[1]・ホアン アパリシオ[1]・滝口正樹[2]・赤星 泉[1]・森 正敬[2]・芳野 信[3]・松田一郎[1]([1]熊大・医・小児科, [2]熊大・医・遺伝研, [3]久留米大・医・小児科). MOLECULAR AND GENETIC STUDIES OF ARGINASE DEFICIENCY : Yougo HARAGUCHI[1], Juan M. APARICIO R.[1], Masaki TAKIGUCHI[2], Izumi AKABOSHI[1], Masataka MORI[2], Makoto YOSHINO[3], Ichiro MATSUDA[1] ([1]Dept. Pediatr., [2]Inst. Med. Genet., Kumamoto Univ. Med. Sch., Kumamoto; [3]Dept. Pediatr., Kurume Univ. Sch. Med., Kurume)

アルギニン血症は肝型アルギナーゼの遺伝的欠損に基づく疾患であり、常染色体劣性遺伝形式を示す。今回、アルギニン血症の1例について遺伝子の構造解析を行った。EcoRIおよびHindⅢで切断したサザンブロット解析では、患者には遺伝子座の大きな欠失や挿入はないことがわかった。患者培養リンパ球DNAよりEMBL4をベクターに用いて患者遺伝子ライブラリーを作製し、肝型アルギナーゼ遺伝子を単離し、塩基配列を決定したところ、第3エクソン内に 4bpの欠失があった。蛋白翻訳領域 262bp-265bpのAAGAまたは、263bp-266bpのAGAAが欠失していると考えられた。いずれの場合にも88番アミノ酸以後でフレームシフトがおこり、132番で終止コドンが現れ、この変異遺伝子の産物にはアルギナーゼ活性はないものと思われる。また、この4bpを含んだ領域を PCR法で増幅し、アクリルアミドゲル電気泳動を行ったところ、2本のバンドを検出した。本患者は Compound heterozygoteであり、この 4bpの欠失した遺伝子は父親由来であることがわかった。

B 39

Gyrate Atrophyの分子生物学的研究：堀田喜裕[1], 藤木慶子[1], 笹部哲生[2], 塩野　貴[3], 早川むつ子[1], 中島　章[1], George Inana[4].（[1]順天堂大眼科, [2]大阪大眼科, [3]東北大眼科, [4]National Eye Institute, NIH, Bethesda MD）
MOLECULAR BIOLOGICAL STUDY OF GYRATE ATROPHY : Yoshihiro HOTTA[1], Keiko FUJIKI[1], Tetsuo SASABE[2], Takashi SHIONO[3], Mutsuko HAYAKAWA[1], Akira NAKAJIMA[1] and George INANA[4] ([1]Dept. of Ophthalmol. Juntendo Univ., Tokyo; [2]Dept. Ophthalmol. Osaka Univ., Osaka; [3]Dept. Ophthalmol. Tohoku Univ., Sendai; [4]National Eye Institute, NIH, Bethesda MD)

Gyrate Atrophy（GA）は常染色体劣性の遺伝形式をとるまれな網膜脈絡膜変性症であるが、オルニチンアミノトランスフェラーゼ（OAT）の欠損が知られていて、生化学的によく解明されている。われわれはGAの原因を遺伝子レベルで知るためにヒトOATのcDNAをプローブにして10人のGA患者の皮膚線維芽細胞を培養して抽出した高分子DNA, mRNAをサザンブロット, ノーザンブロットを行い検討した。1人の患者にEcoR Ⅰ, Bgl Ⅱ, Hind Ⅲ, Msp Ⅰで正常と異なったパターンがみられ、対立遺伝子の片方のOAT遺伝子の部分欠失が考えられ、またこの患者のOATのmRNAは検出できなかった。この患者ではOATのmRNAが全くないことが原因でOATが作られないと考えられる。さらにこの患者の遺伝子ライブラリーを作りスクリーニングをして、とったOAT遺伝子の解析を行っている。その他の9例ではサザンブロット、ノーザンブロットではみかけ上正常な点突然変異等と考えられgenetic heterogeneityが予想される。つまりこの疾患はOAT遺伝子の異常によって引き起こされるがその異常はさまざま（例、欠失、点突然変異など）と考えられた。

B 40

ヒトOAT cDNA probeによるNorrie病のDNA診断：藤木慶子[1], 堀田喜裕[1], R. G. Weleber[2], W. H. Murphey[2], M. Litt[2], 早川むつ子[1], 中島　章[1], G. Inana[3].（[1]順天堂大眼科, [2]Oregon Health Sci. Univ., Portland OR, [3]National Eye Institute, NIH, Bethesda MD）
DNA DIAGNOSIS OF NORRIE DISEASE BY HUMAN OAT cDNA PROBE : Keiko FUJIKI[1], Yoshihiro HOTTA[1], Richard G. WELEBER[2], William H. MURPHEY[2], Mike LITT[2], Mutsuko HAYAKAWA[1], Akira NAKAJIMA[1] and George INANA[3] ([1]Dept. of Ophthalmol., Juntendo Univ., Tokyo; [2]Oregon Health Sci. Univ., Portland OR; [3]National Eye Institute, NIH, Bethesda MD)

10q26とXp11.2にマップされるオルニチンアミノトランスフェラーゼ（OAT）はミトコンドリアマトリックス酵素で、その欠損は高オルニチン血症をきたし、常染色体劣性の遺伝形式をとるまれな網膜脈絡膜変性症の一種であるgyrate atrophyをひきおこす。X染色体上に存在するOAT関連遺伝子の機能は不明であるが、X染色体性の網膜色素変性症のDNAマーカーとして注目されるL1.28（DXS7）と同じ位置に存在する。

我々はNorrie病の1家系について、ヒトOAT cDNAをプローブとして種々の制限酵素を用いてgenomic DNAのSouthern blot analysisを行なった。結果はSca Ⅰで消化した時、6kbpのX染色体由来のRFLP（restriction fragment length polymorphism）がNorrie病と連鎖し、Norrie病の座位がOAT関連遺伝子Xp11.2に近接することが示唆された。またRFLPによって保因者であることが予想された。

B 41

ヒトプロリダーゼ：その遺伝子構造の解析およびプロリダーゼ欠損症での遺伝子異常.
田上昭人・遠藤文夫・松田一郎（熊大・医・小児科）. HUMAN PROLIDASE : GENE STRUCTURE OF HUMAN PROLIDASE AND IT'S MUTATIONS : Akito TANOUE, Fumio ENDO, Ichiro MATSUDA (Dept. Pediatr., Kumamoto Univ. Med. Sch., Kumamoto)

ヒトプロリダーゼ遺伝子を解析した。遺伝子の大きさは140kb以上と非常に大きく、15個のエクソンから構成されていた。最も短いイントロンは、第3イントロンの1.0kb，最も長いのは第9イントロンで50kb以上に及んでいることが明らかになった。5'上流部の解析では、primer extension の結果 cap siteが intiation codonの上流部 153bpのところに存在し、さらに cap titeより43bp上流部に CAT boxが存在した。また、エクソン・イントロン結合部は全て GT/AG ruleに従っていた。

プロリダーゼ欠損症患者由来の細胞 8例について酵素活性，CRMの有無，蛋白合成能，mRNAの有無を調べた結果、全て活性は低下もしくは消失しており、2例がCRM，蛋白合成能，mRNAが陽性で、残りは陰性であった。CRM陽性の 2症例は、第12エクソンの point mutationで276番目のアミノ酸が Aspより Asnに変化していることが判明した。また、CRM陰性の一症例は、第14エクソンの部分を含む deletionであることが明らかになった。

B 42

cDNA CLONING AND GENOMIC ANALYSIS OF HUMAN GLYCINE DECARBOXYLASE:
Akihiro KUME[1], Shigeo KURE[2], Keiya TADA[1], Kuniaki NARISAWA[2], and Koichi HIRAGA[3] ([1]Dept. Pediatr. and [2]Dept. Biochem. Genet., Tohoku Univ. Sch. Med., Sendai; [3]Dept. Biochem., Toyama Med. Pharmac. Univ., Toyama)

Glycine decarboxylase (GDC) catalyzes the first step of glycine cleavage reaction, and most cases of nonketotic hyperglycinemia (NKH) are caused by GDC deficiency. These patients lacked an immunoreactive peptide (apparent molecular weight was about 100 kDa), which was detected in control human livers with an anti-chicken GDC antibody. Using this antibody and chicken GDC cDNA fragments, human GDC cDNA was cloned and verified. The obtained cDNA had a total length of 3,783 bp and covered complete coding region, whose open reading frame encoded 1,020 amino acids. Homology between huamn and chicken GDC was 83%.

We performed Southern blot analysis on genomic DNAs prepared from 15 normal individuals with the cDNA, and RFLPs were detected when the DNAs were digested with MspI, PstI, or TaqI. In addition, Southern analysis on genomic DNA preparations from eight NKH patients revealed that one patient with GDC deficiency had a partial deletion of the gene. The human GDC cDNA should be useful in linkage analysis of the pedigrees involved with GDC deficiency or abnormality.

B 43

MOLECULAR CLONING OF cDNA CLONES FOR $E_1\alpha$, $E_1\beta$ AND E_2 COMPONENT OF HUMAN BRANCHED CHAIN α-KETOACID DEHYDROGENASE COMPLEX AND SOUTHERN BLOT ANALYSIS OF MUSD PATIENTS.

Yoshitaka NOBUKUNI, Hiroshi MITSUBUCHI, Fumio ENDO and Ichiro MATSUDA (Dept. Pediatr., Kumamoto Univ., Kumamoto)

A deficiency of branched chain α-ketoacid dehydrogenase complex (BCKDH) results in maple syrup urine disease (MSUD). BCKDH comprises E_1 (decarboxylase), E_2 (transacylase) and E_3 (dihydrolipoamide dehydrogenase) components, and the E_1 component is further composed of subunits $E_1\alpha$ and $E_1\beta$. Previously, We reported the defects of $E_1\beta$ and E_2 in MSUD patients by immunoblot analysis. In order to elucidate the molecular mechanisms of MSUD, We isolated and characterized the cDNA clones for $E_1\alpha$, $E_1\beta$ and E_2 components for human BCKDH. The deduced primary structures show that mature $E_1\alpha$, $E_1\beta$ and E_2 of human BCKDH are composed of 400, 342 and 421 amino acid residues, respectively. Genomic DNA from normal and 4 lymphoblastoid cell lines from CRM for $E_1\beta$ negative patients gave the same restriction maps on southen blot analysis.

B 44

HETEROGENEITY OF ENZYME DEFECTS AND THE CLINICAL EXPRESSION IN 3-KETOTHIOLASE DEFICIENCY.

Seiji YAMAGUCHI, Hiroyuki NAGASAWA, Toshiyuki FUKAO, Tadao ORII (Dept.Ped.,Gifu Univ.,Gifu) and
Takashi HASHIMOTO (Dep.Biochem.,Shinshu Univ.,Matsumoto)

3-Ketothiolase deficiency is an inherited disorder of isoleucine and ketone body catabolism. A considerable degree of heterogeneity of expression has been described. We studied about correlations between clinical features and the enzyme defects on five patients using immunochemical methods. Case 1 (male): Before severe attack of ketoacidosis, his development was normal. Case 2 (female): She had suffered from frequent attacks and her development was retarded. Case 3 (male) : He had suffered from several attacks because of the disease but was well-developed. Case 4 (male) : His development was also normal although he had several episodes of ketoacidosis. Case 5 (male) : He is the father of case 4 and had no symptoms. Fibroblasts from the above patients lacked the thiolase activity due to mitochondrial acetoacetyl-CoA thiolase. In pulse chase experiments, mutant enzyme proteins were detected in all patient's fibroblasts and they were various in amount, life-span or molecular sizes. However, there were found no correlations between the enzyme defect patterns and the clinical features in these patients.

B 45

MOLECULAR CLONING OF cDNAs FOR HUMAN MITOCHONDRIAL 3-HYDROXYACYL-CoA DEHYDROGENASE AND 3-OXOACYL-CoA THIOLASE. Akira OHTAKE, Hiroki ABE, Hiroo NIIMI (Dept. Pediatr., Chiba Univ., Chiba), Masaki TAKAYANAGI (Dept. Metab., Chiba Children's Hosp., Chiba), Yoshinori SATOH, Shigenori YAMAMOTO (Dept. Pediatr., Shimoshizu Natl. Hosp. and Sanatorium, Yotsukaido, Chiba), Yoshihiro AMAYA, Masaki TAKIGUCHI and Masataka MORI (Inst. Med. Genet., Kumamoto Univ., Kumamoto)

Mitochondrial 3-hydroxyacyl-CoA dehydrogenase (HADH)(EC 1.1.1.35) and 3-oxoacyl-CoA thiolase (T1)(EC 2.3.1.16) catalyze the third and fourth steps of mitochondrial fatty acid β-oxidation system, respectively. Inherited deficiencies of these enzymes may be misdiagnosed as Reye's syndrome, sudden infant death syndrome, etc. To facilitate investigation of the enzymes and gene structures and to elucidate the nature of the mutation in inherited disorders of fatty acid oxidation, we isolated cDNA clones for human HADH and T1. Oligo d(T)-primed and random primer human liver cDNA libraries in λgt11 were screened using isolated rat HADH and T1 cDNAs as probes. Three positive clones cover more than 85% of the HADH mRNA sequence. Three of positive clones for human T1 contained an overlapping cDNA sequence with an open reading frame encoding a polypeptide of 397 amino acid residues (predicted Mr, 42,053). The predicted amino acid sequence of human T1 is 87% identical with those of the rat T1. RNA gel blot analysis of human liver RNA showed a single mRNA of 1.7 kilobases for HADH and 1.6 kilobases for T1, respectively.

B 46

A complementation study of peroxisome-deficient disorders.
Yasuyuki SUZUKI, Shigehiro YAJIMA, Nobuyuki SHIMOZAWA, Tadao ORII (Dept. Pediatr., Gifu Univ., Gifu), Takashi OSUMI, Takashi HASHIMOTO (Dept. Biochem., Shinshu Univ., Matsumoto)

Peroxisome-deficient disorders, which include Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), are fatal inherited metabolic diseases. The primary etiology of absent peroxisomes and genetic relationship among these diseases have not been elucidated. We investigated the genetic heterogeneity of peroxisome-deficient disorders by a complementation study using somatic cell fusion technique. Formation of peroxisomes in the fused cells was evaluated by immunofluorescence staining using anti-catalase IgG and FITC-conjugated second antibody. Fibroblasts from 11 patients with ZS, 4 with NALD and 3 with IRD were divided into 5 groups: group A: ZS (n=3) and IRD (n=1); group B: ZS (n=1); group C: ZS (n=3); group D: ZS (n=1); group E: ZS (n=3), NALD (n=4) and IRD (n=2).

These results indicate that ZS, the severest phenotype of peroxisome-deficient disorders, has 5 different genotypes. On the other hand, one defective gene product may lead to wide range of clinical manifestation. Also these data suggest that at least 5 gene products are required for functional peroxisomes.

B 47

HIGH FREQUENCY OF HOMOALLELIC ^{444}LEUCINE TO PROLINE MUTATION IN THE NON-NEURONOPATHIC FORM OF GAUCHER DISEASE. Mitsuo MASUNO, Shunji TOMATSU, Kazuko SUKEGAWA and Tadao ORII (Dept. Pediatr.,Gifu Univ. Sch. Med., Gifu)

A ^{444}leucine to proline mutation detected by a Nci I polymorphism in the human glucocerebrosidase gene was studied to investigate the correlation of the three clinical phenotypes of Gaucher disease with this mutation in 11 Japanese patients with Gaucher disease (type Ⅰ, 8 patients; type Ⅱ, 1 patient; type Ⅲ, 2 patients) and to determine the feasibility of the use of genomic probe DNA for carrier detection and prenatal diagnosis in 8 Japanese families with Gaucher disease and agreeable to family study (type Ⅰ, 6 families; type Ⅲ, 2 families). The homoallelic ^{444}leucine to proline mutation was found only in patients with type Ⅰ (non-neuronopathic form) disease. Of the 8 type Ⅰ patients, 5 had the homoallelic mutation and 2 had one mutant allele. One patient with type Ⅱ disease was not of this mutant allele. Of the 2 type Ⅲ patients, one had a single mutant allele whereas the other exhibited no mutation of this kind. These findings seem to conflict with others showing that this mutation is partially responsible for the occurrence of neuronopathic Gaucher disease. Thus, the Nci I polymorphism will not be useful for the diagnosis of subtypes of Gaucher disease. Carrier detection was feasible in three families with type Ⅰ disease of the 8 families analyzed by the Nci I polymorphism.

Masuno, M. et al. 1989. Hum. Genet. in press.

B 48

Gaucher病の遺伝子解析：ゲノム遺伝子の解析とfunctional geneの選択的増幅による^{370}Asn→Serの検出．辻省次[1]・宮武正[1]・大和田操[2]・Edward I. Ginns[3]（[1]新潟大・脳研・神経内科，[2]日大駿河台病院・小児科，[3]National Institute of Mental Health）．
MOLECULAR GENETICS OF GAUCHER DISEASE:ANALYSIS OF GLUCOCEREBROSIDASE GENOME AND DETECTION OF ^{370}Asn→Ser BY SELECTIVE AMPLIFICATION OF GLUCOCEREBROSIDASE FUNCTIONAL GENE:Shoji Tsuji[1], Tadashi Miyatake[1], Misao Owada[2] and Edward I. Ginns[3] ([1]Dept. Neurol., Brain Res. Inst., Niigata Univ., Niigata; [2]Dept. Pediatr., Surugadai-Hosp., Nihon Univ., Tokyo; [3]Clin. Neurosci. Branch., National Institute of Mental Health, Bethesda)

Gaucer病は，ライソソーム水解酵素の一つであるグルコセレブロシダーゼの遺伝的欠損によってその基質であるグルコセレブロシドが蓄積するスフィンゴリピドーシスの一つである．本疾患の分子レベルでの解析を進めるためにまずグルコセレブロシダーゼ遺伝子の詳細な解析を行った結果，グルコセレブロシダーゼをコードしているfunctional geneに加えて相同性の極めて高いpseudogeneが存在することが示された．そのため通常の遺伝子増幅法(polymerase chain reaction, PCR)を行ったのでは両方の遺伝子が増幅されてしまいfunctional gene内の変異の検出が困難であった．塩基配列の解析から，このpseudogeneは，functional geneと同様のエクソン，イントロンの構造をとっていて，そのエクソン９内に，55bpのdeletionが存在することを利用して，deletion部位の塩基配列からプライマーを合成し，さらにこの変異をはさむように上流のイントロンからもう一つのプライマーを合成してＰＣＲによりfunctional geneのみを選択的に増幅し，oligonucleotideを用いたallele-specific hybridizationの手法により容易に^{370}Asn→Serの変異を検出する方法を確立した．

B 49

ANALYSIS OF β -GALACTOSIDASE DEFICIENCY

Akihiro Oshima, Michie Shimmoto, Yukiko Fukuhara, Yoshiro Nagao, Yoshiyuki Suzuki, Hitoshi Sakuraba (Dept. Clin. Genet., Tokyo Metr. Inst. Med. Sci., Tokyo)

Complete or partial deficiency of lysosomal β -galactosidase (β -gal) activity has been observed in various human inherited metabolic diseases. Fibroblasts obtained from patients with GM1-gangliosidosis (F-GM), Morquio B disease (F-MB) and galactosialidosis (F-GS) are studied to clarify genetic relationship of β -gal deficiency diseases. Expression of human β -gal cDNA in F-GM and F-MB led to an increase in β -gal activity, but not in F-GS. Expression of human protective protein cDNA in F-GS led to an increase in β -gal activity, but not in F-GM or F-GS. Abnormal size of β -gal mRNA was observed in one case of F-GM and an amount of protective protein mRNA was reduced in several cases of F-GS. These findings support the previous reports that GM1-gangliosidosis and Morquio B disease are caused by mutation of β -gal gene and galactosialidosis is by mutation of protective protein gene.

B 50

FABRY DISEASE: IDENTIFICATION AND CHARACTERIZATION OF POINT MUTATIONS USING GENE AMPLIFICATION.

Michie SHIMMOTO, Akihiro OSHIMA, Yukiko FUKUHARA, Yoshiyuki SUZUKI and Hitoshi SAKURABA (Dept. Clin. Genet., Tokyo Metr. Inst. Med. Sci., Tokyo)

Fabry disease is an X-linked recessive disorder that results from a deficiency of α -galactosidase A(α -Gal A). Our prelimirary data suggest a heterogeneity of mutations in this disease, with the preponderance of single DNA base substitutions. We have adapted and extended the polymerase chain reaction (PCR) to detect Fabry mutations by DNA sequencing of PCR-amplified α -Gal A cDNA. The entire nucleotide suquence was identical with the originally reported sequence of normal human α -Gal A cDNA except for a single nucleotide substitution for G_{131} to A in family O, and from G_{902} to A in family M. The mutation results in amino acid changes, Trp_{44} to Stop, and Arg_{301} to Gln. In addition, the mutation in family O produced new Nhe I site in exon 1. The identification of hemizygotes and heterozygotes was perfomed successfully by Southern blot analysis. The application of these procedures should facilitate the investigation of a wide variety of inherited metabolic disease.

B 51

HIGH FREQUENT RFLPs AT THE 5' END OF THE DMD GENE DETECTED BY cDNA.

DENG Han-Xiang, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

New RFLPs detected at the 5' end region of the cDNA (cDMD-1a) of the Duchenne muscular dystrophy (DMD) gene are reported. In 42 phenotypically and karyotypically normal female persons (84 X chromosomes) studied, at least 5 PvuII-fragments were detected. Of the 5 fragments, 4 (two pairs) are polymorphic, consisting of a 10kb (A1-allele)/7.4kb(A2-allele) RFLP and a 13kb(B1)/4.6kb(B2) RFLP. Allele frequencies calculated from the 84 X chromosomes are 0.43, 0.57, 0.97, and 0.03 for A1, A2, B1, and B2 alleles, respectively. Five different genotypes are expected in each RFLP in the general Japanese population. The high-frequent RFLPs in this region of the DMD gene are useful not only for family analysis of DMD, particularly for prenatal and/or carrier diagnosis, but also for the study on the parental origin of X chromosome abnormalities. Using these RFLPs as genetic markers, we could ascertain the parental origin in four such cases.

B 52

MOLECULAR GENETIC ANALYSIS OF THE FAMILIES AFFECTED WITH DUCHENNE MUSCULAR DYSTROPHY

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The molecular genetic analysis was carried out in 17 X-linked muscular dystrophy individuals (15 Duchenne [DMD] and two Becker [BMD] type) by using the dystrophin cDNA. We have detected 12 deletions, corresponding to a deletion rate of 71%. The deletions were distributed in two regions, clustering primarily near the center of the gene and secondarily near the 5'region.

Tweleve deletions belonged to 10 families. Carriers were identified in four DMD and one BMD families by using densitometer scanning. We found novel junctional fragments of the carriers in three families. New mutation of the affected male was diagnosed in one family. Carrier state was not clear in the other one family.

B 53

MOLECULAR GENETIC AND IMMUNOPATHOLOGICAL ANALYSIS OF DYSTROPHIN IN TWO PATIENTS WITH COMPLEX GLYCEROL KINASE DEFICIENCY.

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DNA analyses and immunopathological studies on two patients with complex glycerol kinase deficiency were described. DNA studies using Duchenne muscular dystrophy (DMD) cDNA and various genomic clones showed that the deletions extended through the 3' end of this gene with the 5' break points corresponding to cDNA segment 10 in both patients. The responsible genes for glycerol kinase and congenital adrenal hypoplasia are estimated to be mapped between L1.4 locus and DMD locus. Although the pathological findings of the muscle biopsy specimens from the patients were nonspecific mild myopathic changes, immunofluorescent analysis with dystrophin antibody clearly demonstrated no staining of dystrophin at muscle surface membranes. These findings suggest that the 3' deletions in dystrophin gene in our patients caused production of incompetent proteins which could not adhere to the surface membrane. Otherwise, the lack of ability for producing normal mRNA might fail to produce corresponding protein.

B 54

走査型電子顕微鏡による染色体の立体視. 飯野晃啓・稲賀すみれ・名黒知徳・八木正樹・亀家俊夫 (鳥取大・医・第1解剖) STEREO SCOPIC OBSERVATION OF CHROMOSOMES BY SCANNING ELECTRON MICROSCOPY : Akihiro Iino, Sumire Inaga, Tomonori Naguro, Masaki Yagi and Toshio Kameie (Department of Anatomy, Tottori University, School of Medicine, Yonago)

染色体の微細構築をより立体的に観察するために、走査電顕像のステレオ撮影を行ない、ステレオペアーで供覧した。

染色体試料には、ヒト培養リンパ球、ＨｅＬａ細胞およびムラサキツユクサ花粉母細胞を３：１メタノール酢酸で固定した後、酢酸処理法および凍結割断法により得られた染色体と、界面展開法によって得られたチャイニーズハムスター骨髄細胞染色体を用いた。ステレオ像は、走査電顕下に傾斜角１０°と２０°で同一視野の写真を２枚撮影することにより作製した。

立体観察の結果、ヒト培養リンパ球およびＨｅＬａ細胞の染色体は、クロマチン基本線維とよばれる直径約３０ｎｍの線維が不規則にからみあって構築されていた。また、チャイニーズハムスターの染色体では、直径約３０ｎｍの線維のほかに約１００ｎｍの線維や、３０ｎｍ線維が更に解きほぐされたと思われるヌクレオソームビーズ構造など染色体を構築する様々な太さの線維が同時に観察された。

一方、ムラサキツユクサでは、減数分裂細胞の割断面に多数の微細小管からなる紡錘糸とコンパクトな染色体の断面が観察された。また減数第一分裂中期の染色体全体像では、直径約４０－５０ｎｍの線維が密に凝縮し、更に太いらせん構造を呈しているのが明瞭に認められた。

B 55

走査型電子顕微鏡による均衡型相互転座の観察. 佐々木裕之[1]・陳　偉業[1]・高林俊文[2]・新宅裕子[2]・矢嶋　聰[2]([1]十和田市立中央病院・産婦, [2]東北大・医・産婦) Reciprocal translocation observed by SEM:Hiroyuki SASAKI[1]Chan WAI IP[1], Tosifumi TAKABAYASI[2], Yuko SINTAKU[2], and Akira YAJIMA[2] ([1]Dept.Obstet.Gynecol., Towada City Hospital.,Towada;[2]Dept.Obst.Gynecol.,Tohoku Univ.,Sendai)

46, ＸＸ, t（6；7）(q25；p15) と46, ＸＸ, t（6；Ｘ）(p25；p11) の2例の均衡型相互転座を走査型電子顕微鏡（以下ＳＥＭ）を使用して観察した。電顕試料作製には標本に厚みをもたせる為，長時間ギムザ染色法を使用した。ギムザ染色をより長時間加えることにより染色体周囲の細胞質様の物質が取り除かれ，従来のＳＥＭ image とは異なる鮮明な染色体が観察可能になった。また光学顕微鏡（以下ＬＭ）標本と同一の核板をＳＥＭで比較できるよう作製法を工夫した。本法によると転座部位は十分な厚みをもって観察可能であり，核型分析能はＬＭに匹敵することが確認された。46, ＸＸ, t（6；7）(q25；p15) の例では転座部位があたかも gap の様に観察され脆弱性を示す転座部位が観察された。このような転座結合部位の詳細が観察可能であることに加えＬＭ標本と同一の核型をＳＥＭで観察できることから本法は今後臨床応用が可能であることが示唆された。

B 56

PARENTAL ORIGIN OF X CHROMOSOME ABNORMALITIES ASCERTAINED BY RFLP STUDIES. DENG Han-Xiang, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Parental origins of X chromosome abnormalities in six cases were traced with RFLP analyses using several X-linked genes (cDNA clones of the DMD gene) or cloned DNAs as probes. Patients studied included 2 cases of 49,XXXXY, one each of 49,XXXXX, 46,X,del(X)(p11), 46,X,dup(X)(p21) and 46,X,i(Xq). In all of 3 numerical abnormalities studied, successive nondisjunctions at maternal meiosis were the responsible mechanism: a nondisjunction at the first meiosis, and subsequent non-disjunctions occurring simultaneously in both homologous chromosomes at the second meiosis. Among 3 structural abnormalities, i(Xq) was of maternal origin, whereas the other two were of paternal origin. These findings may indicate that numerical aberrations of X chromosome result in general from nondisjunction at maternal meiosis, while structural aberrations occur through paternal meiotic errors, except for isochromosome. Our conclusion is not inconsistent with the previous hypothesis that structural aberrations other than Robertsonian translocation are preferentially of paternal origin.

B 57

DISOMIC HOMOZYGOSITY IN TRISOMY 21: A MECHANISM RESPONSIBLE FOR TRANSIENT MYELOPROLIFERATIVE SYNDROME (TMS). Kyohko ABE, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki), Naoki Harada (Cytogenet. Res. Depart., Kyushu Med. Sci., Fukuoka), Yoshimitsu FUKUSHIMA (Div. Med. Genet., Saitama Child. Med. Cen., Saitama), Tadashi KAJII (Dept. Pediat., Yamaguchi Univ. Sch. Med. Ube)

TMS is a leukemoid reaction frequently found in newborns with Down syndrome (DS) and rarely in phenotypically normal infants (NDS). We studied 12 (9 DS and 3 NDS) TMS patients cytogenetically. Proliferating cells in TMS stage are all 21-trisomic or tetrasomic cells, regardless of phenotypes, suggesting that additional copies of a gene in chromosome 21 play an important role in the occurrence of TMS. Heteromorphism analysis of chromosome 21 with QFQ/RFA-banding in the trisomic cells revealed that 2 of 3 chromosomes 21 are identical, showing an "aab" heteromorphic pattern, i.e., disomic homozygosity. This indicates that additional #21(s) had resulted from second meiotic or somatic nondisjunction. Assuming a mutant allele (t) of the putative gene, TMS cells of the patients would be disomic-homozygous "Ttt", while either of the parents would be a "Tt" heterozygote. We propose disomic homozygosity of a mutant allele as a mechanism responsible for the occurrence of TMS. This can be tested by an RFLP analysis in DS families using #21-spanning probes, and the gene locus would be proximal to a crossing-over site.

B 58

Parallel ELEVATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY AND CHROMOSOME DAMAGE IN CULTURED CHROMOSOME INSTABILITY SYNDROME (CIS) CELLS. Syuiti ABE[1], Kuwang-Ho LEE[2] and Michihiro C. YOSHIDA[1] ([1]Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo, and [2]Dept. Biol., Korea Univ., Seoul, Korea)

The basal levels of SOD activity and chromosome aberration (CA) and sister chromatid exchange (SCE) frequencies were examined in cultured fibroblasts or EBV-transformed lymphoblastoid cell lines (LCLs) from CIS patients with Bloom's syndrome (BS), Fanconi's anemia, or ataxia telangiectasia (AT), and in those from embryos and normal subjects served as controls. Although LCLs tended to exhibit a higher background SOD level than fibroblasts due to an elevation of Cu/Zn-SOD activity, BS and FA fibroblasts with increased frequencies of CAs and/or SCEs showed an abnormally elevated baseline SOD activity, particularly Mn-SOD level, compared with control cells. However, BS and AT LCLs with almost control levels of CA and SCE frequencies showed no or a slightly elevated SOD activity, suggesting a possible selection of such cells during EBV-transformation. The observed parallelism between SOD activity and chromosome damage implies an involvement of active oxygen species, especially superoxide radicals, in the increased chromosome damage of CIS cells.

B 59

DNA TEMPLATE AND DEOXYNUCLEOSIDE EFFECTS ON HIGH FREQUENCIES OF SISTER CHROMATID EXCHANGE (SCE) IN BLOOM SYNDROME (BS) CELLS AND A MUTANT CELL LINE ORIGINATED FROM ATAXIA TELANGIECTASIA (AT). Yukimasa SHIRAISHI and Ming-Jie LI (Dept. Anat., Kochi Med. Sch., Kochi)

The effect of bromodeoxyuridine (BrdU)-substituted DNA template and thymidine (dT) pool on the excess SCEs was studied in BS and an AT-derived mutant cell line (AsHa). When BS endomitotic cells were labeled with low and high BrdU concentrations during S_1 and S_2, only the BrdU concentration during S1 phase affected the high SCE frequency. SCE highly decreased to about half level in AsHa cells labeled with various BrdU doses (40, 60, 80 and 100 μg/ml) during only S_1, compared with those labeled during S_1 and S_2. Co-cultivation of AsHa and BS cells resulted in a significant reduction in SCE level from 70 to 13-17 SCE level in BS cells, lowered the BrdU concentrations necessary for sister chromatid differential (SCD) staining from 40 to 10 μg/ml with normal SCE level and resulted in highly decreased level of SCEs under high BrdU doses (100 μg/ml)(12-14 SCE) in AsHa cells, compared with originally increased SCE (36.65 SCE at 100 μg/ml). The basis of the very high BrdU doses necessary to achieve SCD and direct measurements of TMP synthetase activity have shown that AsHa cells have a high level of TMP synthetase, which keeps a large pool size of dT. These findings strongly support that excess and decreased dT pools are closely related to the condition necessary for the high SCE induction.

B 60

LEVELS OF SISTER CHROMATID EXCHANGES (SCEs) IN HYBRID CELLS BETWEN BLOOM SYNDROME B-LYMPHOBLASTOID CELLS AND VARIOUS CELL LINES WITH LYMPHOID MALIGNANCY. Hirotsugu KOBUCHI (Center for Adult Diseases, Kurashiki) and Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Kochi)

The present study has been undertaken to examine the effect of cell hybridization of Bloom syndrome (BS) B-lymphoblastoid cell lines (LCLs) and various cell lines with lymphoid malignancy in order to clarify the relationship between sister chromatid exchange (SCE) and malignant conditions, since most carcinogens have been known to cause SCE at some level following carcinogen treatment (possibly during carcinogenesis). Cell hybridization study has shown that though BS high SCEs were complemented by fusion with normal cells, various malignant cell lines could not have resulted in complete normalization of BS SCEs, retaining 15-30 SCEs per hybrid cells, demonstrating possibly common defects in DNA in BS and malignant cells. These findings strongly support that some character of high SCE in BS cells has some connection to the malignant condition in that at least one step in carcionogenesis is either accompaneid by the production of SCE, or that SCEs themselves cause such step to occur.

B 61

A NEW MUTANT RETAINING HIGH SISTER CHROMATID EXCHANGE AND HIGH BLEOMYCIN SENSITIVITY FROM ATAXIA TELANGIECTASIA B-LYMPHOBLASTOID CELL LINE. Ming-Jie LI and Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Kochi)

The chromosome aberrations and sister chromatid exchanges (SCEs) were examined in 4 ataxia telangiectasia (AT) derived B-lymphoblastoid cell lines (B-LCLs)(AT-S, AT-SHI, AT-SHI B13A and AsHa) following treatments with neocarzinostatin (NCS) and bleomycin. All of these cell lines exhibited extremely high frequencies of chromosome aberrations with the treatments of NCS and bleomycin. Among them, AsHa, a mutant B-LCL originated from an AT patient, showed high frequencies of SCEs under high bromodeoxyuridine (BrdU) concentrations retaining hypersensitivity to NCS and bleomycin with regard to chromosome aberrations. A clear BrdU dose-dependent increase of SCEs (9.85 SCEs/cell at 40 μg/ml, 36.65 SCEs/cell at 100 μg/ml on average) in this mutant was observed. When AsHa mutant cells were treated with NCS (0.02 μg/ml) and/or bleomycin (5.0 μg/ml) under 40 μg/ml BrdU (minimum BrdU concentration for sister chromatid differential staining), SCE levels increased from 9.85 SCE baseline level to 21.1 SCE with NCS and 20.5 SCE with bleomycin, respectively, with dose dependent manner. These observations indicate that AsHa is a unique AT derived mutant cell clone with high SCE character retaining original hypersensitivity to bleomycin and NCS.

B 62

EXPRESSION SPECIFICITY OF AND SCE INDUCIBILITY AT THE EB-VIRUS MODIFICATION SITE-1 (11q23) IN LYMPHOBLASTOID CELL LINES: Tatsuro IKEUCHI (Dept. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ.), Yoshiko TERUI (Dept. Biol., Fac. Educat., Waseda Univ.) and Kohtaro YAMAMOTO (Dept. Virol. Immunol., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The EBV-virus (EBV) modification site-1 (EBVM1, 11q23.1) is expressed as isochromatid gaps by BrdU treatment (50-100 μg/ml, 24 hrs) specifically in B-lymphoblastoid cell lines (LCLs) established by EBV mediated transformation (Sutherland et al. 1987; HGM9, 1987). However, genetic implications and expression mechanism of EBVM1 have remained unknown. Using a total of 20 LCLs of different sources, we obtained the following results concerning the specificity of EBVM1 expression and inducibility of sister-chromatid exchanges (SCE) at this site. 1) EBVM1 was found in all the LCLs established by EBV infection with expression rates of 20-90%, but not in either EBNA-positive LCLs spontaneously established or B-lymphocytes cultured for 3-4 days after EBV infection. 2) Mean SCE numbers per chromosome 11 showing a gap at 11q 23 were not significantly different from the expected values based on the ratio of the length of normal chromosome 11 to that of a whole genome. Further, the sites of SCEs were uniformly distributed along the entire length of chromosome 11. The findings indicate that the EBVM1 is not a hot spot for intrachromosomal recombination as measured by SCEs.

B 63

CHROMOSOME ABERRATIONS OF HUMAN SPERMATOZOA INDUCED BY IN VITRO EXPOSURE TO TRITIATED WATER.

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The effects of tritium (HTO) β-rays on human sperm chromosomes were studied using our interspecific in vitro fertilization system between human spermatozoa and zona-free hamster oocytes. Nine semen samples from 5 healthy men were treated with media containing 1.53-24.3 mCi/ml HTO for about 80 minutes. Twelve hundred and ninety spermatozoa from the controls and 1,842 spermatozoa from the irradiated groups were successfully karyotyped.

The incidence of spermatozoa with radiation-induced structural chromosome aberrations increased linearly with increasing dosage, reaching 72.8 % at 1.93 Gy of β-rays. Breakage-type aberrations occurred far more frequently than the chromatid-type aberrations. Chromosome-type aberrations appeared far more frequently than the chromatid-type aberrations. All of these types of aberrations showed linear dose-dependent increases. The relative biological effectiveness (RBE) of HTO β-rays relative to γ-rays was calculated for the above-mentioned 5 indices, respectively. Their RBE values ranged from 0.94 to 1.36.

B 64

INDUCTION OF STRUCTURAL CHROMOSOME ABERRATIONS IN CHINESE HAMSTER SPERMATOZOA BY X- AND γ-IRRADIATION.

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Male Chinese hamsters were exposed to 0.91, 1.82 and 3.63Gy X-rays and to 1.10, 2.15 and 2.95Gy γ-rays. They were mated with females having marker chromosomes within 5 days after the irradiation. Two hundreds and seven spermatozoa from the control, 387 spermatozoa from the X-ray groups and 403 spermatozoa from the γ-ray groups were karyotyped.

Incidences of spermatozoa with radiation-induced chromosome aberrations were 14.7, 23.4 and 52.1% in the X-ray groups and 14.0, 33.8, and 42.6% in the γ-ray groups. The incidences increased linearly with increasing dosage in both groups. The relative biological effectiveness (RBE) of X-rays relative to γ-rays ranged between 0.9 and 1.1, indicating that both kinds of radiation were very similar in their capacity of inducing chromosome aberrations in spermatozoa of this species. Most of chromosome aberrations induced by X- and γ-rays were of chromosome-type. Incidences of aberrations of breakage-type increased linearly, while those of exchange-type increased quadratically. The latter included inversions and reciprocal translocations. Some of these aberrations were also found in 12.5-day embryos and 18.5-day fetuses.

B 65

THE AGE-RELATED CHANGES OF MUTAGEN SENSITIVITY IN THE MOUSE SPLEEN LYMPHOCYTES. Hitoshi HOSHINO, Tatuya TAKESHITA, Sumio IIJIMA, Akio ASAKA (Dept. Health Sci., Yamanashi Med. Col., Yamanashi) and Makoto HIGURASHI (Dept. Maternal and Child Health, Univ. Tokyo, Tokyo)

To investigate the age-related changes of mutagen sensitivity, spleen lymphocytes from young (14-16 weeks) and old (111 weeks) male BDF1 mice were cultured with either EMS, MMS, MNNG, HN_2, MMC, or 4NQO in the presence of RPMI 1640, heat inactivated FBS, LPS, 2-ME and BrdUrd for the analysis of SCE. Though all 6 mutagens induced significant dose-related increases in SCE frequency, there were no significant differences between young and old mouse lymphocytes.

The same lymphocytes were cultured after X-irradiation with the same medium without BrdUrd for the analysis of chromosomal aberration (dicentric +ring) to investigate the age-related changes of X-ray sensitivity. The spontaneous aberration frequencies in old mice were significantly higher ($p<0.05$) than those in young group. However, there were no differences in the X-ray induced aberrations between young and old mouse lymphocytes. There were some reports which indicated the age-related decline of DNA repair ability after DNA damage by carcinogens. But the present studies were not consistent with those reports.

B 66

X-RAY INDUCED SCEs AND CHROMOSOMAL ABERRATIONS IN LYMPHOCYTES FROM PATIENTS WITH KLINEFELTER SYNDROME.
Zentaro YAMAGATA, Sumio IIJIMA, Tatuya TAKESHITA, Toshimi OOMA, Akio ASAKA (Dept.Hlth Sci.Yamanashi Med.Col.,Yamanashi), and Makoto HIGURASHI (Dept.Matanal and Child Health, Univ. of Tokyo,Tokyo)

The chromosomal sensitivity to X-ray in cell from patients with Klinefelter syndrome,characteristic of sex chromosomal disorders and high risk of malignant tumor,was studied by techniques of sister chromatid exchanges (SCEs). Heparinized peripheral blood samples were obtained from four patients with Klinefelter syndrome and four karyotypically normal controls. The blood samples were exposed to X-ray at doses of 0. 2.5, 5.0 and 7.5 Gy. Immediately after the irradiation, medium and PHA were added to each culture. The frequencies of X-ray induced SCEs increased in proportion to the increase of X-ray doses. At all X-ray doses there were more frequencies of SCEs in cells from patients than from controls but not significant statistically. The frequencies of dicentrics and rings per cell in Klinefelter syndrome at dose of 7.5 Gy was significantly increased than in cells from normal controls ($p=0.0478$).

B 67

HIGHER INDUCTION OF CHROMOSOME ABERRATIONS IN FIBROBLASTS FROM PATIENTS WITH POROKERATOSIS. Tatsuya TAKESHITA, Kayo SHIMIZU, Zentaro YAMAGATA, Sumio IIJIMA, Akio ASAKA (Dept. Health Sci., Yamanashi Med. Col., Yamanashi) Makoto HIGURASHI (Dept. Maternal Child. Health, Univ. Tokyo, Tokyo) Fujio OTSUKA (Dept. Dermat., Univ. Tokyo, Tokyo)

Porokeratosis is an autosomal dominant inherited disorder. The lesions are characterized by localized abnormal keratinization. High occurrence of malignant tumors from the lesions have been reported. Recently, higher sensitivity to X-irradiation was found in the fibroblasts derived from the porokeratosis patients. In the present study, baseline and x-ray induced chromosome-type aberrations were investigated in fibroblasts from the patients and controls. No aberrations were found in the cells from the two groups without X-irradiation. As for exchange-type aberrations (dicentric plus ring), after 1.5 Gy of X-ray, aberration frequencies were 0.20 ± 0.05 and 0.27 ± 0.03 for the patients and controls, respectively. After 3.0 Gy of X-ray, those were 0.52 ± 0.10 and 0.43 ± 0.04, respectively. With regard to deletion-type aberrations, after 1.5 Gy, frequencies in the patients (0.71 ± 0.15) were higher($p=0.057$) than those of the controls (0.29 ± 0.03). Likewise, after 3.0 Gy of X-ray, frequencies in the patients (1.45 ± 0.10) were higher($p=0.091$) compared to the controls (0.96 ± 0.15).

B 68

SOME FACTORS RELATING TO TWINNING.
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(3) The junior high school affiliated to the Tokyo Univ., Tokyo

We analyzed some factors relating to twinning. The data were got by 373 twin candidates to the junior high school affiliated to the Tokyo University, from 1981 to 1989, and their parents. Their zygosity was determined by many genetic markers or answers of convenient questionnaire for zygosity diagnosis. Among the distributions of the maternal and paternal age, age difference between parents, and the interval between marriage and twin birth, the difference based on zygosity of index twin pair was clearly recognized as to maternal age. Namely the ratio of DZ twinning was higher than that of MZ when maternal age was over 30s. Moreover, the more the parity increased, the more the ratio of DZ twinning were apt to increase. As to the familial incidence of twinning, 44% (117/266) of index MZ and 46% (49/107) of index DZ had twin relatives, suggesting that twins are more common among the relatives of twins than in the general population. The percentage of twin relatives of maternal side was nearly the same as that of paternal side in index MZ, but the percentage of maternal side was much higher than that of paternal side in index DZ. These results suggests that twinning is related to maternal condition especially concerning DZ twinning.

B 69

HOW DO THE MOTHERS OF TWINS THINK OF ZYGOSITY DIAGNOSIS?

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(3) The Japanese Association of Twins' mothers, Tokyo

Questionnaire on zygosity diagnosis was sent to the members of The Japanese Association of Twins' Mothers. The questions are as follows: Do you hope zygosity diagnosis of your children? Do you think zygosity diagnosis to be important or not? Have you ever been told your children's zygosity, and where were you told so? Moreover convenient questionnaire for zygosity diagnosis which we have developed was included in the questionnaire. We have received 564 answers till now. The results was as follows. 40.0% (193/482) of mothers wanted zygosity diagnosis. 52.0% (270/519) of mothers thought zygosity diagnosis to be important. And 489 of mothers have the experience to be told that their children's zygosity might be MZ or DZ, and 92.0% (450/489) was told so at obstetrical hospital. According to the answers of convenient questionnaire for zygosity diagnosis, it was revealed that more than 90% of twins told to be MZ were correctly diagnozed, however, only 40% of twins told to be DZ were correctly diagnosed on the contrary. These results suggests that many number of MZ must be mistaken for DZ by means of only obstetrical findings such as placenta and so on.

B 70

HEALTH LIFE-STYLES AND HEALTH SITUATIONS IN ADULT TWINS.

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Health life styles (HLS)and health situations in adult twins were studied inorder to clarify to genetic and enviromental factors. The data were got by 123 pairs of twinors (20-54 years old, MZ=109 DZ=10 PZ=4),who graduated from the junior high school affiliated to the Tokyo University. There are 41 questions about life styles(reference to Breslow's health life style), physical health situations(PHS) (modifications of Dean's scale) and mental health situations(MHS) (modifications of Zung's SDS). Mean of HLS (smoking, drinking, breakfast, sleep, free time, exercise and nutritional balance) was significantly lower in males(3.81 ± 1.58)than in females (5.09 ± 1.00).The correlation coefficient between points of HLS and age was 0.0082 (ns). Intraclass correlation coefficient of HLS among MZ was 0.272 ($P<0.001$) but that among DZ was 0.191 (ns). Likewise, intraclass correlation coeficients of PHS and MHS among MZ were 0.588 ($p<0.001$) and 0.328 (0.001),and among DZ, those were 0.047 (ns) and 0.184 (ns),respectively. In MZ those intraclass correlation coefficients except MHS were significant, whether they were living together or not.

B 71

BONE MINERAL DENSITY AND AORTIC PULSE WAVE VELOCITY IN ADULT TWINS. Tatsuya TAKESHITA, Hitoshi HOSHINO, Zentaro YAMAGATA, Sumio IIJIMA, Akio ASAKA (Dept. Health Sci. Yamanashi Med. Col., Yamanashi) Tetsuro NAKAMURA, Yasuyoshi OUCHI, Hajime ORIMO (Dept. Geriatrics, Univ. Tokyo, Tokyo)

Osteoporosis and arteriosclerosis develop during aging process. Both genetic and environmental factors are considered to be involved in the occurrence of the two diseases. In the present study, bone mineral density (BMD), an index for osteoporosis, and aortic pulse wave velocity (PWV), an index for arteriosclerosis of aortic artery, were measured in adult twins to determine genetic and environmental factors related to the two indices. BMD of total body (BMDT) and BMD of lumbar spine (BMDL) were measured using dual energy X-ray absorptiometry (Lunar DP-X). PWV was measured using PWV-200 (Fukuda Electronics). Correlation coefficients between age and the three indices were, among males, -0.2051(N=11), -0.6168 (N=10,$p<0.10$), 0.7336(N=10,$p<0.05$), for BMDT, BMDL, and PWV, respectively. Likewise, among females, those were 0.3396 (N=34, $p<0.05$), 0.1561(N=29), and 0.2597(N=30), respectively. Intraclass correlation coefficients of BMDT, BMDL, and PWV were 0.8019 (N=22), 0.6234(N=22), and 0.6795(N=19), and all were significant ($p<0.001$).

C 1

GENE FREQUENCIES OF ELEVENTH POLYMORPHISMS IN HUMAN SALIVA OF THE EASTERN JAPANESE. Shigenori IKEMOTO, Shuichi TSUCHIDA, Eiji KAJII and Emiko NOZAWA (Dept. Legal Med. and Hum. Genet. Jichi Med.Sch., Tochigi)

The distributions of the gene frequencies ofeleventh polymorphic systems in human parotid and whole saliva were studied in the Japanese population of the eastern part of Japan. The gene frequencies were 0.214 for Pa^{+}, 1.00 for Pb^{1}, 0.753 for Pr^{1}, 0.035 for Db^{+}, 0.394 for PmF, 0.028 for Ph^{+}, o.733 for PIF^{+}, 0.011 for $Amy_1{}^{V}$, 0.217 for $s\text{-}AcP^{A}$. Restriction fragment lengh polymorphism of human salivary amylase after cleavage with restriction endonuclease *Pst I* and *Bam HI* was studied in a population from eastern Japan. Among 40 unrelated individuals, the frequencies of the 5.7 and 6.5 Kbp fragment allele were 0.487 and 0.513, respectively. The gene frequency of Se blood group system observed was 0.516. In the salivary blood substance, protein and enzymes polymorphisms there were differences within the Japanese population, and the salivary polymorphisms appear to be a valuable personal identification, paternity test and anthropological markers.

C 2

GENETIC POLYMORPHISM OF FACTOR I IN THE JAPANESE POPULATION: DESCRIPTION OF A RARE VARIANT. Shigeki NAKAMURA, Akiko SAWAGUCHI (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Polyacrylamide gel isoelectric focusing (PAGIEF) of neuraminidase treated EDTA plasma samples at pH 7-9 with 1.0M urea followed by semi-dry horizontal electroblotting with enzyme immunoassay was done for the detection of Factor I (FI) phenotypes in 604 unrelated Japanese blood donors living in Tokyo. In these samples three common types and one new variant were observed. Three common types were A, AB and B, and a new variant which had major bands in more acidic region than A was designated FI A1. Family data was in accordance with the Mendelian inheritance. The allele frequencies were estimated as $FI^{*}B=0.8924$, $FI^{*}A=0.1068$, and $FI^{*}A1=0.0008$, respectively. The FI protein level of various phenotypes were measured using the single radial immunodiffusion technique. The mean FI proteinlevel for FI B was slightly higher than that for FI A ($p<0.001$) and that for FI A1B ($p<0.05$). The Factor I polymorphism showed a higher variation in the Japanese (mongoloids) than in the caucasoids. So, the FI system is a useful genetic marker for human genetics and anthropological studies.

C 3

GENETIC POLYMORPHISM OF OROSOMUCOID (ORM) IN OKINAWA WITH REFERENCE TO ORM2*Q0

Isao YUASA (Dept. Legal Med., Tottori Univ. Sch. Med. Yonago)
Kazuo UMETSU (Dept. Forens. Med., Yamagata Univ. Sch. Med., Yamagata)

The ORM polymorphism in 364 Japanese from Okinawa was investigated using isoelectric focusing followed by immunoprinting. Twenty-two ORM phenotypes determined by 6 ORM1 and 8 ORM2 alleles were observed. The ORM1*1, ORM1*2, ORM1*2-1, ORM2*5-2, ORM2*1 and ORM2*6 were polymorphic just like those in other Japanese populations. Four new rare ORM2 variant alleles, ORM2*17 ~ ORM2*20 were identified. A sample with ORM1 2 phenotype was completely lacking in ORM2 alloprotein. This pattern was suggestive of ORM2*Q0, probably arisen from unequal crossing-over. A rare ORM2 17 phenotype was likely to result from a heterozygous combination with ORM2*Q0. Its frequency was calculated, using Bernstein's equation, to be 0.054. Assuming that all ORM2*6 and ORM2*Q0 were in association with ORM1*2, the 22 observed phenotypes could be explained by 13 ORM haplotypes.

C 4

GENETIC POLYMORPHISM OF HUMAN PLASMINOGEN : A NEW ABNORMAL PLASMINOGEN WITH A FUNCTIONAL DEFECT.

Masayoshi YAMAGUCHI, Syuichi DOI, Masao YOSHIMURA (Dept. Legal Med., Kinki Univ. Sch. Med., Osaka-Sayama)

Human plasminogen(PLG) polymorphism was investigated by polyacrylamide gel isoelectric focusing followed by immunoblotting. In 5735 plasma samples from unrelated healthy Japanese individuals, four new variants were detected and tentatively designated PLG A^{Osaka}, PLG B^{Osaka}, PLG M^{Osaka} and PLG A^{Nara}. The new variant PLG M^{Osaka} was found in the plasma of two unrelated healthy females in the PLG A-M^{Osaka} and PLG M5-M^{Osaka} phenotypes. The plasminogen activities and concentrations were 52.0 % and 9.1 mg/dl for PLG A-M^{Osaka}, and were 21.0 % and 8.4 mg/dl for PLG M5-M^{Osaka}. It is well known that PLG M5 is an abnormal plasminogen with an inactive molecules. The new variant PLG M^{Osaka} had a very low activity and appears to be an abnormal plasminogen with a functional defect similar to that of PLG M5. It was difficult to detect the electrophoretic patterns of PLG M^{Osaka} and PLG M5 on the gel by a functional assay with a caseinolytic overlay because of their loss of the plasminogen activity. The immunoblotting technique is very useful for detecting inactive molecules such as PLG M^{Osaka} and PLG M5.
The population study showed 20 different phenotypes controlled by 11 different alleles.

C 5

COMPLEMENT ALLOTYPES IN JAPANESE PATIENTS WITH BUERGER'S DISEASE.

Kenji MIZUTANI[1], Hiroaki NISHIMUKAI[2], Naomi KAWATA[1], Katsuyuki OHNISHI[1], Kanji IWAHASHI[1], Kengo TSUNEKAWA[1], Takaaki SHINOMIYA[2] and Hajime KITAMURA[3] ([1]1st Dept. Surg., [2]Dept. Legal Med., Ehime Univ., Ehime; [3]Dept. Immunol., Cent. Adult Diseases Osaka, Osaka)

The allotypes of C3, C6, C7 and factor B (BF) of the complement system were examined in sera from Japanese patients (n=22) with Buerger's disease (thromboangiitis obliterans: TAO) by high voltage agarose gel electrophoresis or agarose gel isoelectric focusing followed by simple protein staining, immunofixation or immunoblotting. There were no significant differences in C3, C6 and BF allele or phenotype frequencies between TAO patients and healthy controls (n=60). The allele frequencies for C7 in the TAO group were as follows (those of controls are given in parentheses). C7*1=0.7727 (0.9000), C7*2=0.0682 (0.0500), C7*4=0.0455 (0.0417), C7*5=0.1136 (0.0083). The C7*5 frequency in TAO group was significantly higher ($0.001<p<0.01$) than that in the controls. A strong association ($0.02<p<0.05$, RR=13.1) was found between TAO and C7 5 phenotype: (C7 5-1)+(C7 5-2)+(C7 5). It appears that C7 5 is an influential etiological factor in TAO.

C 6

COMPLEMENT ALLOTYPES IN PATIENTS WITH BULLOUS PEMPHIGOID AND WITH PEMPHIGUS VULGARIS. Hiroaki NISHIMUKAI, Takaaki SHINOMIYA (Dept. Legal Med., Ehime Univ., Ehime), Koji SAYAMA, Yuji SHIRAKATA, Satoshi SHIRAISHI and Yoshiharu MIKI (Dept. Dermatol., Ehime Univ., Ehime)

We studied the allotypes of C6, C7, factor B (BF) and factor I (IF) of the complement system in serum or EDTA-plasma samples from Japanese patients with bullous pemphigoid (BP; n=14) or pemphigus vulgaris (PV; n=9), by using agarose gel electrophoresis or agarose gel isoelectric focusing. The incidences of the complement allotypes and allele frequencies in the patient groups were different from those in a healthy control group (n=60). In the BP group, higher frequencies for C6*B2 (=0.1071) and C7*5 (=0.0357) were found than those in the control group (C6*B2=0.0417, C7*5=0.0357). In the PV group, the frequencies for C6*B2 (=0.1111), C7*2 (=0.1667) and IF*A (=0.0583) were higher, and those for C6*B (=0.2777) and BF*F (=0.0556) were lower than the frequencies in the control group (C7*2 =0.0500, IF*A=0.0583, C6*B=0.4833, BF*F=0.1917). A very rare C7 allotype, C7 8, was found in a PV patient. It is suggested that PV is genetically and etiologically different from BP.

C 7

GENETIC EPIDEMIOLOGY OF CHRONIC DISEASES- A MULTIVARIATE ANALYSIS OF SERUM URIC ACID. Motofumi MASAKI (Dept. Hygiene, Showa Univ., Tokyo)

A multivariate path model was applied to genetic and cultural inheritance of serum uric acid for a family data set in a small island. This simple model includes 2 parameters to be estimated from a total of 4 correlations among the relatives. The goodness of fit was tested by three models; the general one where both genetic and environmental variance were considered, genetic one with assumption of equal environmental variance, and the environmental with equal genetic variance. In spite of a small sample size, the genetic heritability(h^2) was significant in both general and genetic model, where values were estimated to be 0.366 and 0.423 in general and genetic model, respectively. Cultural heritability(c^2) is significant only in environmental model(0.136). The difference of value of chi-square between general and environmental model indicated that some modifications of original data will be required before entering the models for establishing genetic heritability of serum uric acid.

C 8

LINKAGE STUDY OF DOMINANTLY INHERITED SPINOCEREBELLAR DEGENERATION. Hidenao SASAKI, Akemi WAKISAKA, Kunio TASHIRO(Dep. Neurol. and Pathol., Sch. Med., Hokkaido Univ., Sapporo), Michihiro, C. YOSHIDA(Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo) and higenori IKEMOTODep. Leag. Med., Jichi Med. Sch., Tochigi)

Both autosomal dominant olivo-ponto-cerebellar atrophy(OPCA, Menzel type) and Holmes' cerebello-olivary atrophy are the progressive neuro-degenerative disorders of adulthood with unknown biochemical defects. In order to determine the genetic locus and possible genetic heterogeneity, linkage study has been performed in 22 OPCA families (216 individuals with 77 patients), and 5 Holmes' ataxia families (59 individuals with 18 affected patients) by using 37 polymorphic markers including HLA. The lod scores were calculated by computer program LIPED with the correction of age-dependent penetrance. In both diseases, although the lod scores obtained were usually below -2 indicating no linkage with these markers, some 6q markers (TCP1, PLG) showed positive scores when Joseph's disease was sub-grouped. We are now undergoing the further linkage studies by using markers on 6q.

C 9

A LINKAGE STUDY ON THE AFFECTIVE DISORDER. Shinichiro NANKO, Noboru TAKAZAWA, Hajime KAZAMATSURI (Dept. Psychiat., Teikyo Univ., Tokyo) Masaru KOBAYASHI, Shinobu GAMOU, Jun KUDOH, Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo) and Toshiyuki FURUSHO (Dept. Clin., Genet., Kyorin Univ., Tokyo)

In 1987, Egeland et al, demonstrated a linkage between the affective disorders and two polymorphic DNA markers, c-Ha-ras-1 and INS, on the tip of the short arm of chromosome 11. Other studies, however, have failed to show this linkage. We have investigated the putative linkage between these DNA markers and the affective disorder, assuming that the disorder segregates as a autosomal dominant trait. Subjects were 12 members of two pedigrees with affective disorder. The diagnoses were based on the Research Diagnostic Criteria of Spitzer compiled using a combination of interviews, a review of the case histories, and the Schedule for Affective Disorders and Schizophenia Lifetime version. DNA samples from members of the pedigree were digested with the restriction enzyme SacI. Hybridization was carried out with DNA probes c-Ha-ras-1 and INS. Probe c-Ha-ras-1 revealed two alleles with SacI giving allelic fragments 4.9kb and 6.2kb. Probe INS hybridized to SacI allelic fragments 5.8kb and 6.8kb. The linkage was ruled out in one family. The linkage was ruled out in the other family, in which all the patients are thought to be heterozygotes. Thus, we found no evidence for a linkage of the affective disorder to the markers on chromosome 11, suggesting genetic heterogeneity in the affective disorder.

C 10

EVENT-RELATED POTENTIALS OF FIRST DEGREE RELATIVES OF SCHIZOPHRENIC PROBANDS. Yoichi KIDOGAMI, Yasuhiro INAYAMA, Katsuhiro TOYODA, Hiroshi YONEDA and Toshiaki SAKAI (Dept. of Neuropsychiatry, Osaka Medical College, Takatsuki, Osaka)

Most of the event-related potentials(ERP) studies in schizophrenia have demonstrated a reduction in P300 amplitude. There have been several discussions concerning which of the two, state or trait, is reflected in the reduction but the answer remains unclear. We therefore, recorded the ERP of the first degree relatives of schizophrenic probands(F group) who were over 45 years of age, exceeding the limit of risk period for schizophrenia, and compared their ERP with those of schizophrenics(S group) and controls(C group). These ERP were recorded by auditory "oddball" paradigm in the Fz, Cz and Pz regions at 2 pm, in order to exclude the factor of diurnal variation. The P300 amplitude of S group was significantly smaller than that of C group. The P300 amplitude of F group was also significantly smaller than that of C group. We found no significant differences in the latency of P300 among the three groups. These results suggest that a small P300 amplitude is a trait marker of schizophrenia.

C 11

RAT DERMATOGLYPHICS AS AN EXPERIMENTAL MODEL OF MULTIFACTORIAL INHERITANCE. Michio OKAJIMA (Dept. Forens. Med., Tokyo Med. Dent. Univ., Tokyo)

Dermatoglyphic traits are considered to be inherited in a manner of multifactorial inheritance. The finding of dermatoglyphic traits in the rat (1985) has enabled experimental approaches to the genetics and development. Since patterns on the palmar interdigital pad III are variable and peculiar to each inbred strain, the author conducted a crossing experiment with two inbred strains, WKS with whorls and ACI with triradial patterns (1986). In the present study, more than 300 rats were obtained by crossing two inbred strains, NIG with concentric whorls and ACI. About three quarters of F_1 and F_2 hybrids possessed whorl patterns. Both groups of progeny yielded by backcrossing and double backcrossing to NIG showed only whorls, except one palm in each group. In contrast, patterns of the backcross progeny to ACI, consisted of cusps, loops and whorls, but only few arches and triradii. In the double backcross progeny to ACI, a third of patterns consisted of arches and triradii, while whorls disappeared. The author is now conducting another two crossing experiments. Analysis of the mode of inheritance will be made including the latter experiments.

C 12

COMPARISON OF THE EFFECTS OF COUNTERSTAINING WITH FLUORESCENT OR NON-FLUORESCENT DYES ON QUINOLINE, PYRIDINE AND BENZOTHIAZOL DERIVATIVES TO HUMAN CHROMOSOMES. Kouichi MAMBA (Dept. Vet. Anat., Yamaguchi Univ., Yamaguchi), Misako GOMI, Mutsuo KITAHAMA (Dept. Legal Med., St. Marianna Univ. Sch. Med., Kawasaki) and Akira UTIUMI (Nat. Chem. Lab. Indust., Tsukuba)

The study was carried out to examine the comparison of the effects of counterstaining with distamycin A, methyl green and crystal violet as non-fluorescent dyes on six quinoline, two pyridine and two benzothiazol derivative fluorescence to human chromosomes. These six quinoline drivatives are α-naphthylaldehyde-2-quinolylhydrazone, β-naphthylaldehyde-2-quinolylhydrazone, phenanthrylaldehyde-2-quinolylhydrazone, 1-(2-quinolyl)-3-anthryl-5-(3,5-disulfonaphthyl)formazane,1-(2-quinolyl)-3-anthryl-5-(2-methoxy-5-sulfophenyl)formazane and 1-(2-quinolyl)-3-anthryl-5-phenyl-formazane,The two pyridine derivatives are 1-(2-pyridyl)-3-anthryl-5-(3,5-disulfonaphthyl)formazone and 1-(2-pyridil)-3-anthryl-5-phenylformazane. The two benzothiazol derivatives are 1-(2-benzothiazolyl)-3-anthryl-5-phenyl-formazane and 1-(2-benzothiazolyl)-3-(2-sulfophenyl)-5-(4-methoxyphenyl) formazane. The pairing of α-naphthylaldehyde-2-quinolylhydrazone or β-naphthylaldehyde-2-quinolylhydrazone with methyl green produced R bands. The pairing of phenanthrylaldehyde-2-quinolylhydrazone with methyl green produced greatly enhanced contrast of paracentromeric regions of chromosome 1 and 9.

C 13

High resolution banding in chromosomes of skin fibroblasts and lymphoblastoid cells: Aphidicolin synchronization and ethidium bromide. Akira KUWANO, Tadashi KAJII (Dept. Prdiatr., Yamaguchi Univ. Sch. Med. Ube)

High resolution banded chromosomes were obtained in cultured skin fibroblasts and Ebstein-Barr virus-tarnsformed lymphoblastoid cells by aphidicolin synchronization followed by inhibition of chromosome condensation by ethidium bromide. Aphidicolin was added 22 h prior to harvest at afinal concentration of 0.4 μM, washed with 2 changes of medium without serum, and further maintained for another 6 h in complete medium. Ethidium bromide was added for the last 1.5 h, 7 μg/ml for skin fibroblasts, and for the last 3 h, 5 μg/ml for lymphoblastoid cells. From 46% to 49% of skin fibroblasts, and from 28% to 34% of lymphoblastoid cell chromosomes were in prometaphase. Gaps and breaks induced by aphidicolin were eliminated by removal of aphidicolin for the last 6 h of culturing.

C 14

EXPRESSIVITY OF A COMMON FRAGILE SITE, FRA(3)(p14), WITH APHIDICOLIN TREATMENT. Motoi MURATA, Mikako OTSUKA, Yukiko HAYAKAWA (Div. Epidemiol., Chiba Cancer Center, Chiba), Ei-ichi TAKAHASHI and Tada-aki HORI (Div. Genet., Natl Inst. Radiol. Sci., Chiba)

Common fragile sites on human chromosomes are known to be expressed under various culture conditions and their expressivity shows a wide inter-individual variation. We attempted to study any responsible factors for it. Chromosome test was performed by 72 hrs of whole blood culture, 1) in folate deficient medium (F1O), and 2) with treatment of aphidicolin (34 μg/ml) for final 24 hrs (AP). Number of fra(3)(p14) was counted in 50 cells. Patients of benign and malignant diseases in Chiba Cancer Center Hospital were studied. Their number was 562 for F1O and 134 for AP. In F1O, slightly higher expression frequencies in male than female, and in younger than older patients, both seem to conform to the more remarkable tendencies in healthy blood donors. In AP, too, significant sex- and weak age differences were noted. Neither factors such as family history of cancer, tobacco smoking and affected diseases were associated with the expressivity. Unfavourable prognosis was correlated only with that of F1O but not in AP. As a whole, the expressivities in both cultures appeared uncorrelated. We may conclude that certain physiological condition of cells rather than variable fragility of the site is responsible for the inter-individual variation.

C 15

TISSUE-AND CELL TYPE-DEPENDENT DIFFERENCE OF THE DISTRIBUTION AND FREQUENCY OF COMMON FRAGILE SITES.
Ichiro MURANO, Akira KUWANO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med. Ube)

Tissue-and cell type-dependent difference was studied of the distribution and frequency of aphidicolin-induced common fragile sites. They included PHA-stimulated peripheral blood T lymphocytes, EB virus-induced B lymphocytes, cultured skin fibroblasts and bone marrow cells during remission of malignant blood diseases. Aphidicolin at a concentration of 0.2μM was added for the last 26 h. Both the distribution and frequency of common fragile sites were different depending on the tissues and cell types studied. The only site common to all the cell types studied was that at 16q23. (Hum Genet 1989 in press)

Differences were also studied in excess thymidine-induced common fragile sites in PHA-stimulated T lymphocytes and cultured skin fibroblasts.

C 16

INDUCTION OF RECESSIVE MUTATIONS IN MOUSE PRIMORDIAL GERM CELLS
-EVALUATION OF HUMAN RISK FROM THE RESULT OF MOUSE EXPERIMENT-.
Tohru SHIBUYA, Naoko HORIYA, Hiroshi MATSUDA and Takumi HARA (Hatano Research Institute, Food and Drug Safety Center, Hadano, Kanagawa)

We carried out a specific locus test on mouse primordial germ cells (PGC) with N-ethyl-N-nitrosourea (ENU). Male and female C3H mice were mated and pregnant females were singly injected with ENU at day 8.5, 10.5 or 13.5 of pregnancy, respectively. The F_1 male or female mice obtained were mated with female or male mice of the tester strain, PW. The mice of the next generation were observed on their coat color and ear shape to evaluate whether or not PGC had been mutated with ENU.

The fertility was always lower in female than in male mice and declined as the development of mouse embryos. Mutations were induced with high frequency at 10.5 day PGC compared with the other stages tested. Cluster mutations were frequently observed from the earlier stages of embryos.

Mouse PGC have been considered to be relatively resistant to radiations on induction of mutations, but the above results suggested that mouse PGC are sensitive to ENU. The development of human PGC require much period than that of mice. Therefore, it might be supeculated that human PGC are more suspective on induction of mutations than mouse PGC to a chemical such as ENU.

C 17

Hypomelanosis of Ito: Cytogenetic Studies in Four Patients.
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Hypomelanosis of Ito is an entity in which hypopigmented streaks are present in a background of relatively hyperpigmented skin, due to the presence of two kinds of melanocytes of different pigment potentials arising early in development and migrating often following the Blaschko's line. The melanocyte mosaicism could arise through chromosomal mosaicism, somatic cell mutation, half chromatid mutation or chimerism.

Two patients with hypomelanosis of Ito and another two patients with what could be called hypermelanosis of Ito (hyperpigmented streaks in a relatively hypopigmented skin) were studied. Three of them showed chromosomal mosaicism in both blood lymphocytes and skin fibroblasts (46,XX/47,XX,+mar; 46,XY/47,XY, +13; 46,XY/47,XY, +18), whereas one was chromosomally normal in both tissues studied. Studies of chromosomal heteromorphism in the patients and their parents ruled out chimerism.

C 18

A CASE OF BEALS SYNDROME. Yuko ENDO, Takashi MATSUOKA, Masaaki TANAKA, Takahisa TSUNO, Yoshio YAGI and Taro AKABANE(Dept. Pediatr., Shinshu Univ., Matsumoto)

He is the first child of non-consanguineous 21 years old father and 24 years old mother. After 39 weeks' gestation, he weighed 3136 g. At birth, dyspnea and tachycardia were noticed and subarachnoidal hemorrhage was shown. He was admitted to our hospital on 9 th day. He showed frontal bossing, retrognathia, left low-set ear, "crumpled ear", left torticollis muscularis, kyphoscoliosis, bilateral inguinal hernias and hydroceles, arthrogryposis multiplex congenita(coxa, knee, elbow), camptodactyly, bilateral simian creases and protruded heels. Dyspnea with cyanosis persisted and hyperventilation therapy was needed for 12 days. No congenital heart anomalies were found and secondary pulmonary fetal circulation with sepsis was diagnosed. When he left our hospital 2 months later, he still showed tachypnea due to his chest deformity. Slight hydrocephalus was seen and Moro's reflex was not apparent. At 9 months, muscular hypotonia became marked. Needle biopsy of femoral muscle showed small rounded muscle fibers with variation in size. No enzyme activity was elevated. At 1 year, his body height and weight were below -3σ and head control was nearly possible. At 19 months, he can sit quite a long time playing and can roll over. Arthrogryposis is improved except for coxa. Muscle tonus of legs are better but patellar tendon reflex is not apparent. Xyphoscoliosis is increasing.

C 19

NEW SYNDROME: PECULIAR FACIES, MENTAL RETARDATION, CATARACT, CLEFT PALATE, KYPHOSIS, AND CRANIOSYNOSTOSIS IN 4 SIBS.

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Patients are 4 sibs (a 5y boy, a 4y girl, a 2y boy and a 7m boy). The parents are phenotypically normal and not consanguinous. They have normal 8y and 7y eldersisters. Clinical manifestations among 4 patients include a peculiar facies (frontal bossing, long palpebral fissures, epicanthus, short nose, anteverted nostrils, long philtrum, carp mouth), mental retardation (DQ: 50), cataract, cleft palate and kyphosis. Bone X-ray films show craniosynostosis of lower portion of coronal suture, kypho-scoliosis and decalcification of long bones. Biochemical analysis of blood and urine, amino acid analysis and chromosome analysis show no abnormalities. As far as we know, the manifestations in these 4 sibs have not been reported previously. This is, probably, a new autosomal recessive malformation syndrome.

C 20

WILLIAMS SYNDROME IN PRESUMABLY MONOZYGOTIC TWINS. Tomoko HASEGAWA, Saori NAKAJYO, Hiroyuki NAKANO, Akihiri SAITO (Shizuoka Children's Hosp. Shizuoka), Shigeyo SAKATA, Yoshimitsu SAITO (Shizuoka Red Cross Blood Center, Shizuoka)

Typical features of Williams syndrome (WS) were found in female twins. Clinical features The twins were born in 1983, after a 41-week gestation to a 29-year-old healthy mother and a 33-year-old healthy father. No family members with WS, inclusive of the parents. Birth weights were 1900g (twin A) and 2270g (twin B). Hypercalcemia was not revealed. Both children showed typical elfin-like faces. As the twins were so alike, persons outside the family tended to mistake A for B. Both had supravalvular aortic stenosis of a similar grade. They were friendly, and a cocktail party manner was exhibited. DQ were 42 (A), and 49 (B). The finger-tip patterns of the children resembled each other: twin A had 7 whorls and 3 ulnar loops, and twin B had 8 whorls and 2 ulnar loops. TFRC were 254 and 243. Twin zygosity Clinical diagnosis of zygosity at birth was DZ. No genetic examination was performed at that time. However, results of blood typing for ABO, MN, Rh, Kidd and of HLA showed no discrepancy between the twins. It is thus highly probable that these twins with WS were monozygotic ($Pr(M) > 0.98$), being consistent with previous genetic considerations of WS. DNA fingerprinting may be necessary in order to confirm this.

C 21

SIGNIFICANCE OF TEMPORARY HIGH VALUE OF ALPHA-FETOPROTEIN (AFP) DURING NORMAL PREGNANCY.
Shozo TAMURA, Yuko SHIRAHATA, Mizuho TAKADA, Rihachi IIZUKA (Dept. Obst. Gynec., Sch. Med., Keio Univ., Tokyo), Yukari YANAGI (Keio Health Counseling Center, Tokyo) Yoshiyuki HIRAISHI (Dept. Microbiol., Sch. Med., Keio Univ., Tokyo), Satoshi HATANAKA (Hatanaka Hosp, Tokyo)

The evaluation of the high value of AFP in amniotic fluid of cases without any fetal anomalies is often difficult because of unknown origin. A mother having had the child with congenital nephrotic syndrome visited to our hospital for the purpose of prenatal diagnosis at the 2nd pregnancy. However, because of the high value of AFP (580,000 ng/ml at the 18 weeks of gestation), D. & C. was carried out. At the next 3rd pregnancy the AFP value was relatively high (61,100 ng/ml at the 17 weeks of gestation) but decreased up to 30,380 ng/ml at 19 weeks of gestation and then finally normal baby was born at full term. According to this case it was suggested that the heterozygosity of pathogenic genes may occur such phenomenon as one of possibilities.

C 22

最近の日本の高年出産の増加の実態とこれに伴う染色体異常妊娠増加の推計について
衛生検査所カリオアナリシス 木下芳広 木下恵子 Recent Increase of Advanced Mat-ernal Age Group and It's Effect for Rates of Chromosome Abnormalities in Japan: Yoshihiro KINOSHITA & Keiko KINOSHITA (KaryoAnalysis Inc.,Tokyo)

厚生省人口動態統計などから、日本の高年出産率（母親の年齢が35才以上の出生数／出生総数）を調べたところ、1976年以降は増加する一方であることが明らかになった。1976年は全国の高年出産総数は 69,062 人（全国の出生総数 1,832,617人）で高年出産率は 3.76 %と最低であった。 ところが12年後の1988年には高年出産総数は 106,055 人（全国の出生総数 1,314,006人）となり、高年出産率は 8.07 %となった。 1976 年から1988年にかけて高年出産率は実に 2倍以上にもなり、また全国の出生総数が 0.717 倍と減少しているのに、全国の高年出産総数でみると 1.536倍に増大している。都道府県別にみると、大都市部と沖縄県で高年出産率が高い傾向が明らかになり、東京都と沖縄県では 10 %を越えている。

出生総数が低下する一方の状況の中で、高年出産総数は1985年からは年間10万人を越えており、その影響は様々な方面に現れてくると思われる。

妊婦が高年齢になるほどトリソミー型染色体異常の発生頻度が高くなることがわかっているので、1976年と1988年とで、高年出産群からの染色体異常妊娠発生数と染色体異常率を推計したところ、1988年の染色体異常妊娠総数は 1,713（均衡型を除くと 1,459）となり、その年の全妊娠に対する35才以上の妊娠から生ずる染色体異常妊娠の割合は 0.0013038 であった。1976年はそれぞれ 1,145（ 979）, 0.000627 であった。

なお推計に用いた母年齢別染色体異常妊娠率は Ferguson-Smith & Yates(1984) を用いたが、我々が経験した約 2,450例の羊水細胞染色体検査から高年出産適応を抽出し母年齢別染色体異常妊娠率表を試作し参考に示した。

C 23

SCREENING FOR CHROMOSOMAL ANOMALIES WITH MATERNAL SERUM AFP.
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(Dept. of Tohoku Univ. Sch. of Med. OB/GYN, Sendai)

A total of 526 genetic amniocentesis were performed from July 1, 1986, to August 31, 1989. In each of these patients, the maternal serum α-fetoprotein (AFP) assay had been done by EIA or RIA immediately before genetic amniocentesis. Amniotic fluid AFP levels were also measured by EIA. The samples were obtained between 15 and 18 weeks' gestation. Gestational dates were confirmed by ultrasound studies. The maternal serum AFP results were expressed as multiple of the median (MOM). The amniotic fluid AFP decreased gradually in mid trimester. The maternal serum AFP increased, the median from 15 to 19 weeks of gestation was 46.2, 63.6, 73.2, 87.4 ng/ml in EIA, and 32.2, 45.0, 50.8, 62.6 in RIA. Three fetuses with chromosomal abnormalities were diagnosed: trisomy 21, 4p trisomy, and trisomy 18 (trisomy 18 was in one fetus of a twin pregnancy; the other was normal). Maternal serum AFP values were 0.41MOM for the fetus with trisomy 21, 0.49MOM for the 4p trisomy, and 1.30MOM for the trisomy 18. On the other hand, maternal serum AFP <0.5MOM in normal pregnancies was 7/298 (2.3%) in EIA, and 8/324 (2.5%) in RIA. Our results are in agreement with the majority of the results in the literature, showing that maternal serum AFP levels are lower in association with autosomal trisomy fetuses.

C 24

染色体異常の疫学的研究（第１報）－１番染色体異常（過剰および欠失）－．川村みや子[1]・石井ふみ代[2]・孟建国[2]・新平鎮博[2]・藤田弘子[2]（[1] 大阪府立身障Ｃ・小児，[2] 三菱油化ＢＣＬ，[3] 大市大・児童保健）．ETIOLOGICAL RESERCH OF CHROMOSOMAL ABNORMALITIES.-PARTIAL TRISOMY AND PARTIAL MONOSOMY OF No.1 CHROMOSOME－：Miyako KAWAMURA,Fumiyo ISHII,Kenkoku MOU,Shizuhiro NIIHIRA,Hiroko FUZITA(Dept.Ped., Osaka Pref.Rehabilitation C.,Osaka; Mitubishiyuka.BCL.Co.,Kyoto; Dept.Child Health.,Sci.Living., Osaka City Univ.,Osaka)

1977年～1988年の12年間に報告された１番染色体部分過剰および部分欠失の文献について疫学的研究を行なった。部分過剰は28家系38人、部分欠失は39家系39人であった。頻度の高い染色体切断点は、過剰、欠失、逆位で共通していた。（p38 q25 q32 q42 q43）遺伝性を有するものは、過剰では転座が多く父保因者が8 家系、母保因者が5 家系であった。転座相手の染色体は3 4 6 10 13 18 21 22番であった。欠失では１例のみ逆位であった。部分過剰は３群に部分欠失は４群に分けることができた。各群において男女差はなく、2500g 以下の低体重児の発生率は特に欠失でたかかった。１か月以内の死亡率は過剰で11～38% 欠失で0 ～14% と過剰で高かった。親の年令は欠失で20才未満の母が５人と多いように思われたた。群別の臨床像はばらつきが多かったが、q42qter の部分過剰および部分欠失群は非常に共通点が多かった。q42terは大頭、前頭突出、縫合開大が特徴でq42qter 部分欠失は小頭、丸顔が特徴的であった。また部分欠失で停留睾丸、尿道下裂が多いのが目立っていた。精神運動発達は、中度から重度と予後は決してよいものではなかった。

C 25

A CASE OF RING CHROMOSOME 7: CLINICAL AND PATHOLOGICAL STUDY.

H. TSUKAMOTO, M. TANIIKE, J. NISHIMOTO, M. MIDORIKAWA, K. INUI, S. OKADA (Dept. Pediatr., Osaka Univ., Osaka), M. NAKATSUKASA, W. YOSHIHARA (Suita City Hosp., Osaka), H. SAKAMOTO, Y. SUGAWARA, H. MIMURA and J. FURUYAMA (Dept. Clin. Genet., Hyogo Coll. Med., Hyogo).

A case of ring chromosome 7 (46,XY,r(7)(p22q36)) was presented. The patient was a second male infant of unrelated healthy parents. No particular family history was recognized. At birth, craniofacial dysmorphism (microcephaly, bilateral cleft lip and palate, hyperterolism, mongoloid slant, epicanthal folds, suddle nose, hypoplastic low set ears and navus flameus on the forehead), several cafe au lait spots on the trunk, and hypoplastic genitalia were noticed. He did not show any mental and motor development and died of pneumonia at 20 months. Autopsy was performed. Macroscopically, no anomalies were found except the brain, which weighted only 290 gm. The hypoplastic frontal lobes were fused with each other at the base, and olfactory nerve and pineal body were not found. Microscopically, Purkinje's cells were found in the cerebellar white matter and melanocytes were found at the cerebellar vessels, suggesting abnormal cell migration. This case is the first report of pathological findings of ring chromosome 7 and important to think the pathogenesis of differential abnomalies caused by chromosomal aberrations.

C 26

A case of inverted chromosome 9 with severe mental retardation and autistic symtom

Yasuhiro INAYAMA, Hiroshi YONEDA, Yasuhiro NONOMURA, Toru ISHIDA, Youichi KIDOGAMI, Katsuhiro TOYODA and Toshiaki SAKAI.
(Dept.Neuropsychiat., Osaka Medical College.Takatsuki, Osaka.)

The proband was a 38 year-old male, who showed delayed speech development at age 1 and became increasingly restless at age 7. He was admitted to a mental hospital at 9 due to autistic, negativistic symptoms and severe mental retardation. No physical anomalies or neurological deficits were found. High resolution chromosome analysis revealed that his karyotype was 46XY inv(9)(p11q13). His father and first sister showed the same karyotype. The first sister had a congenital dislocation of hip joints, but no other physical abnomalities. The second sister died after convulsive seizures at age 3. The father had no physical, mental or neurological abnormalities.

It has been reported that an inverted chromosome 9 is related to mental retardation, autism and physical anomalies, however, it has not been proven that chromosome 9 inversion produce any pathognomonic features. This anecdotic case suggest that this chromosome aberration may cause a variety of symptoms such as congenital dislocation of hip joints, mental retardation and autistic symptoms.

C 27

A CASE WITH AN INTERSTITIAL DELETION IN THE LONG ARM OF CHROMOSOME 9.

Hiromi SAKAMOTO[1,2], Osamu MIKAMI[1,2], Masafumi HANDA[1,2], Yoshie SUGAWARA[2], Hiroko MIMURA[2], Jun-ichi FURUYAMA[1,2] ([1]Dept. of Genet. and [2]Clin. Genet., Hyogo Col. of Med., Nishinomiya)

A boy was born on March 12, 1987 to the first child of 26-year-old father and 27-year-old mother. Mother experienced threaten abortion at 7-month-gestation. He was born at 40-week-gestation after uncomplicated delivery. He weighted 3600g at birth. His left hip joint was dislocated, for which he had an operation at 11-month-old. On the examination at 18-month-old, he was noticed his speech was poor and could not walk. He could hold his neck at 4-month-old and could roll over at 7-month-old. He was referred to us for cytogentical examination at 19-month-old. His height was 79.2cm and his weight was 9.7kg. He showed hypertelorism, arched eyebrows, thick lower lip, large but simple ears, and low-set ears. We revealed his karyotype was 46,XY,9q- and his parents' were normal. His chromosome 9 had de novo interstitial deletion in the long arm. His karyotype was decided as 46,XY,del(9)(q31.1q33.2). Pure monosomy of the long arm of chromosme 9 is very rare. We can find only 6 cases. The deleted regions of thses cases are different from ours and the clinical features in our case do not correspond to those reported peviously.

C 28

t(15;16)(q15;q13). A TRANSLOCATION THROUGH THREE GENERATIONS IN FIVE CARRIERS IN A FAMILY WITH UNTREATED PKU SISTERS. Mashio KITATANI, Mamoru OZAKI, Etsuko TAKASE and Hiroaki TAKAHASHI(Clini. Genet. of Hum. Genet., Med. Res. Inst., Kanazawa Med. Univ., Ishikawa)

A number of cases of phenylketonuria (PKU) have been reported, but very few cases have been described with familial translocation. We report a case of a reciprocal translocation t(15;16)(q15;q13). This chromosome constitution was found in the sisters with PKU, their mother , the first child with maternal PKU of elder sister and an abortus of sister. They had many spontaneous and therapeutic abortions before and after giving birth to mentally retarded children. The second child of elder sister and the first child of sister with maternal PKU had normal karyotyps. Unbalanced translocation and or recombinant were not found in this family. The sisters were the first and the third product of healthy and non-consanguineous parents.

C 29

A case of partial 12p11.2→pter Trisomy, that had been diagnosed with 21/21 translocated 21 trisomy.

Naoki NOMOTO, Yuri MIYANOMAE, Kazuhisa ISHIMURA (Dept. Pediat., Kyoto City Child Welfare Center, kyoto), Osamu NAGAUCHI (Dept. Clin. Labo., kyoto City Hosp., Kyoto)

We reported a 1 year old female with peculiar features, poor feeding and developmental retardation, who was not diagnosed as 21/21 trancelocated 21 trisomy but trisomy for the short arm of chromosome 12.

Proband was the product of full term pregnancy born to a woman(gravida 1, para 0). She was born when her mother was 25 year old and father 24 year old. Her common features included low birth weight, poor feeding, hypotonicity, sever developmental and mental retardation, large head, protruding forhead, small eyes, hypertelorism, short nose, small mouse, high arched palate, small low set ears. Dermatoglyphic patterns was right simian crease, bilateral normal palmar axial triradii, worl of left first fingertip pattern, and urnal loops of the other fingertip patterns, and L^{t} of bilateral hallucal areas. Her weight was 2850g at birth and 7150g at 1 year old, length 47.6cm at birth and 68.5cm at 1 year old, head circomference 32.0cm at birth and 44.2cm at 1 year old, and chest circumference 31.0cm at birth and 43.5cm at 1 year old.

Using G-banding method, the mother's karyotype was 46,XX,t(12;21)(p11.2;p11.2). Proband's karyotype was 46,XX,-21,+der(21)t(12;21)(p11.2;p11.2)mat. The father's karyotype was normal. In the low banded chromosome, ?/21 trancelocated chromosome was similar to 21/21 trancelocated chromosome or isochromosome of 21 chromosome.

C 30

A MALFORMED GIRL WITH PARTIAL MONOSOMY 21: DOES FULL MONOSOMY 21 EXIST IN LIVE-BORNS? Nobuko TOMITA, Naoki HARADA (Cytogenet. Res. Dept., Kyushu Med. Sci., Fukuoka), Kyohko ABE, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

A female infant with partial monosomy for 21pter-q22.1 is reported. She has dolichocephaly, antimongoloid slant of palpebral fissures, depigmented iris, short nose, fish-like mouth, micrognathia, low hair-line, short neck, wide-set nipples, anal atresia, pes varus, and developmental retardation. Chromosome analyses with GTG- and QFQ-bandings performed in her 200 peripheral blood lymphocytes and 100 skin fibroblasts had suggested full monosomy 21, but RFA- and high-resolution bandings revealed a translocation between 2q37.3 and 21q22.1. The parents' karyotypes were normal.Thus patient's karyotype is interpreted as 45,XX,-2,-21,+der(2),t(2;21)(q37.3;q22.1). Gene dose analysis on slot-blots using 4 cloned DNAs on #21 as probes confirmed her partial monosomy. There is a hypothesis that full monosomy 21 does not exist in liveborns. Of previously reported monosomy 21 liveborns, 8 were said to have full monosomy. However, chromosome analyses on the cases were not thoroughly performed, and some of the cases who had seemed full-monosomic was found to have partial monosomy 21 with further R-/T-banding analyses. Moreover, full monosomy 21 is very rare even in spontaneous abortuses. The finding in our patient, together with those in previous studies, supports the hypothesis.

C 31

A CASE OF FRAGILE(X) SYNDROME WITH ELEVATED SERUM TESTOSTERONE. Kazumi Ikawa, Miki Yazima, Junko Nakatuka, Hiroko matuzaki, Emiko Nakayama (Ishikawa Health Service Association) Tamotsu Sato, Noboru Igarashi (Dept. Pediatr., Kanazawa Univ., Ishikawa) Shigeru Maruyama (kanazawa Holly Spirit Hosp., Ishikawa) Hiroko Kawashima (Wazima Public Health Center)

We present a case of Fragile(X) with elevated testosterone. a 4-year-old boy was refered to us because of severe delay in speech development and hyperactivity. His parents and sisiter have normal intelligence, but his mother has a mentally retarded cousin. His clinical findings include mental retardation, short stature (-2.1σ), mildly dysmorphic large ears, and macro-orchidism (3.0ml). Fragile(X) expression from culture with MTX was 11% in patient,and 2% in his mother. Endocrinological test revealed an elevated serum testosterone(44.4ng/dl), with low LH response to LH-RH stimulation. SM-C level was also elevated.

A slightly elevated testosterone with macro-orchidism might be clinical markers of Fra(X) in childhood.

C 32

STUDIES ON COMPUTERIZED TOMOGRAPHY OF XYY SYNDROME Yasuhiro NONOMURA, Hiroshi YONEDA, Yasuhiro INAYAMA, Toru ISHIDA, Takashi TSUJI, Katsuhiro TOYODA, Shigetoshi TSUTSUMI, and Toshiaki SAKAI
Department of Neuropsychiatry, Osaka Medical College, Takatsuki, Osaka

Pneumoencephalographic studies on XYY syndrome have been done by Asaka et al., Hakola et al., and Hashi and Usui et al. They found a ventricular enlargement of mild or moderate degree. There have been only few reports on the studies of computerized tomography in XYY syndrome. We add here three cases of XYY syndrome combined with schizophrenia or schizophrenia-like psychosis by use of the computerized tomography. We found in one case an enlargement of lateral ventriculi. It may be assumed that an extra Y chromosome may be related to the enlargement of the lateral ventriculi.

C 34

CHARACTERISTICS OF BREAKPOINTS IN CHROMOSOME ABNORMALITIES OBSERVED IN MYELOCYTIC LEUKEMIA AND RELATED DISORDERS. Masako MINAMIHISAMATSU, Takaaki ISHIHARA (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba)

In 309 cases of myelocytic leukemias and related disorders studied for the past four years, 1985 through 1988, the distribution of breakpoints of their structural chromosome abnormalities except those of specific translocations such as t(9;22), t(8;21) and t(15;17) was investigated. Such chromosome abnormalities were observed in 171 of 309 cases. The number of the breakpoints related to the chromosome abnormalities in the 171 cases amounted to 191, consisting of 108 by translocations, 66 by deletions, 8 by isochromosomes, 6 by inversions and 3 by duplications. The breakpoints 1q32, 7p11, 11q23 and 12p13 were mainly related to translocations, 7q22, 9q22 and 20q11 to deletions, 2q33, 5q13 and 12p11 to both translocations and deletions, and 17p11 to isochromosomes. In the translocations the partner chromosomes of the highly occurring breakpoints were not limited to specific chromosomes. The breakpoints showed no specific relations with types of the diseases. The results of the investigations reported here as well as at the previous annual meeting seem to suggest that most of the breakpoints may be related to the progression rather than to the genesis of the diseases.

C 35

CYTOGENETIC STUDY FOR TRANSLOCATION 8;21 TYPE LEUKEMIA

Tomoe TAKAHASHI, Yasunobu YOKOYAMA, Yuki OOTA, Midori OOTAKE, Ichirou KAMINO, Hiroko MATSUI, Yoshimori ISHIHARA, Kazumasa HIKIJI, and Yutaka TSUKADA (Dept. Genet. Res. Lab. SRL Inc. Tokyo)

Cytogenetic studies were performed for 188 cases of t(8;21) type leukemia. Standard and complex translocations which involving 8q22, 21q22 and other chromosome were detected in 186 of 188 cases. Chromosome aberations additional to standard and complex 8;21 translocation were most frequently observed were Y and/or X missing(in 65.1%) and del(9)(in 9.1%). And also, interstitial deletion, del(2)(q34q37) and trisomy 4 as minor additional chromosome abnormalities were observed in four cases each. Extra derivative chromosome, +der(8) and +der(21) were detected in two cases of t(8;21)(q22;q22) type leukemia. Complex translocations were found and conserved the direction of translocation, 21q22-ter to 8q22, which constructed the critical recombinant chromosome in all eleven cases that supporting Rowley's idea. Simple variant translocation which involved 21q22 and 8q24 instead of 8q22 were found in other two cases. And one case of them were shown that three way rearrangements between 8q24, 21q22, and a third translocation partner. These variant types were classified as M2-FAB subtype.

C 36

DETECTION OF THE REARRANGEMENT WITHIN THE FIRST INTRON OF ABL GENE IN CHRONIC MYELOGENOUS LEUKEMIA AND PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA.

Hikari NISHIGAKI, Shinji TANAKA, Hitoshi NAKAGAWA, Shigeo HORIIKE, Masafumi TANIWAKI, Shinichi MISAWA, Chihiro SHIMAZAKI (Dept. Int. Med., Kyoto Pref. Univ. Med., Kyoto), Johji INAZAWA and Tatsuo ABE (Dept. Hygiene)

The Philadelphia chromosome (Ph) resulting from a 9;22 translocation, t(9;22)(q34;q11), characterizes malignant cells in more than 90 % of patients with chronic myelogenous leukemia (CML), and in 25-33 % of those with acute lymphoblastic leukemia (ALL). To identify the breakpoints in ABL gene on chromosome 9 (band q34) directly, we performed long-range mapping of ABL using pulsed field gel electrophoresis. Hybridization of v-abl probe to SfiI digests revealed germline (225kb) and rearranged bands in six of ten patients with Ph+CML, in one with Ph+ALL, and in two of three with Ph-bcr+CML. In the remaining five patients, the rearranged bands were detected when SfiI (or NotI) digests were hybridized to T39-1-2 and p5'IV probes, which are derived from the extreme 5' end of ABL gene. These data indicate that chromosome 9 breakpoints in patients can be mapped between ABL exon Ib and II.

C 37

DETECTION OF DNASE I SENSITIVE REGIONS ON FIXED HUMAN CHROMOSOMES BY IN SITU NICK TRANSLATION WITH BIOTINYLATED dUTP

Masafumi TANIWAKI, Keiichi MIYAGOE, Teruyuki TAKASHIMA, Yukari SHIZUMI, Hikari NISHIGAKI, Shigeo HORIIKE, Shinichi MISAWA, Kei KASHIMA (Dept. Med., Kyoto Pref. Univ. Med., Kyoto), Toru SUGIMOTO (Dept. Pediat.), Johji INAZAWA, Tatsuo ABE (Dept. Hygiene)

Fixed chromosomes prepared on slides according to routine methods were labeled by nick translation using biotinylated dUTP. DNase I nicking sites, where bio-11-dUTP was incorporated, were visible by subsequent binding of avidin-biotin-peroxidase complex or fluorescein avidin DCS. Nonradioactive labeling is less time consuming and offers a better resolution of the labeling patterns comparing with autoradiographic procedures. We found a pattern of alternative DNase I sensitive and insensitive regions. The DNase I sensitive regions tend to correspond to R-bands, although some dark G-bands are DNase I sensitive. The telomeric regions of the chromosomes are strongly labeled. HSRs obtained from neuroblastoma cell line RT3 were highly sensitive to the DNase I digestion: 10ng/ml DNase I completely destroyed all structures of the HSR. However, in situ nick translation demonstrated no differential staining patterns on the HSRs. The procedure will be modified allowing to study fine structural and functional organization of chromosomes.

C 38

CYTOGENETIC STUDY OF RENAL CELL CARCINOMA.**Tatsumi OHHASHI [1], Jiro ISHIKAWA [2,3], Kei-ich UMEZU [2], Sadao KAMIDONO [3], Shuntaro NAKAGAWA [4], Yoshimori ISHIHARA [1], Kazumasa HIKICHI [1], Yutaka TUKADA [1] ([1] SRL INC, Tokyo ; [2] Dept Urol., Kobe Nat. Hosp., Kobe ; [3] Dept. Urol., Kobe Univ. Sch. Med., Kobe ; [4] Dept. Med., Hyogo Med. Center for Adults., Kobe)**

In this meeting, we present the cytogenetic studies for 13 cases of Renal cell carcinoma (RCC) and its 8 cases of normal tissues. Cytogenetic examination was performed by short-term (2-14days) culture with enzymatically disaggregation. Clonal chromosomal abnomalities, numerical or stuructual aberations on #3 chromosome, were deteced in nine of ten RCC cases (includes 2 treated cases) except four cases which showed the Fibroblast liked cell growthed in primary cultures, successively. This findings strongly support the idea that #3 chromosomal aberation is the most common chromosomal abnormality and a primary rearrengement in RCC.

Moreover, chromosomal studies were performed on normal kidney tissues whith obtained from eight RCC patients. -Y or +7 as chromosomal aberation in one cell at the minimum were obsrved 7/7 and 7/8 cases respectively. However, the both numerical aberation was observed only one case in RCC tumor tissues. Therfore, it is suggested that -Y or +7 as sole numerical aberation which present in normal kidney tissues of RCC patient not be relating its carcinogenesis.

C 40

EXTRACTION OF GENOMIC DNA FROM FORMALIN-FIXED TISSUE. Sei OKIMOTO, DENG Han-Xiang, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

We report a method for the extraction of genomic DNA from long stored, formalin-fixed tissues. Several grams of the formalin-fixed liver, spleen, kidney, lung or muscle were laid in tap water for 7-10 days, minced well, washed with 0.85% NaCl, and suspended in PBS. After the suspension was incubated with agitation at 60°C for 2-3 hrs, the tissue fragments were suspended in 500mM Tris·HCl (pH 9)/20mM EDTA/ 10mM NaCl. The suspension in which 1% SDS and 1 mg/ml proteinase K were added was incubated with agitation at 50 C for 24 hrs, and for 50 hrs with addition of another SDS and proteinase K solution. Genomic DNA was extracted with the phenol/chloroform/ethanol method. After measuring the concentration, the DNA underwent Southern hybridization using P20 (locus:Xp21.2) as a probe. The results showed that (1) the longer storing period, the smaller size of DNA was obtained, (2) the DNA with a relatively high-molecule appropriate for the Southern analysis could be extracted from the tissues stored by 2 years, and (3) the genomic DNAs extracted from the 1-2 year-stored tissues of a female showed clear Southern blots showing 14kb and 7.5kb fragments. The present method is applicable for the family analysis of a genetic disease and the detection of an oncogene. Even if a small size of DNA is obtained, it is also useful when the PCR technique is combined.

C 41

MOLECULAR ANALYSIS OF FEMALE PATIENTS WITH cDNA PROBES OF DUCHENNE MUSCULAR DYSTROPHY. Fumiko SAITO, Kohtaro YAMAMOTO* and Akira TONOMURA (Dept. Cytogenet., *Dept. Virol. Immunol., Tokyo Med. Dent. Univ., Tokyo)

Duchenne muscular dystrophy (DMD) is an X-linked recessive and genetically lethal disorder, affecting approximately 1 in 3,300 males. The entire 14-kb cDNA of the DMD gene has recently been cloned, and its protein product, called dystrophin, has been identified. Most of the DMD mutations are intragenic deletions detected in 50-70% of the male patients studied. In this report, we present the molecular analysis of the female patients with male DMD within their families.

Genomic DNA from 7 families involving 7 female and 3 male patients was isolated from their EBV-transformed lymphoid cell lines and digested with Hind III, Southern transferred, and hybridized to cDNA probes. The autoradiograph produced by the cDNA (8) probe shows that a deletion was detected in one female patient and her mother among these seven families.

C 42

ISOLATION OF HUMAN Y CHROMOSOMAL DNA FRAGMENTS.
Masato TSUKAHARA, Shinya MATSUURA, Fumio KISHI, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube) and Akira YOSHIDA (Dept. Bioch. Genet., Beckman Research Institute of the City of Hope, Duarte)

We screened a flow-sorted human Y chromosomal library (ATCC No. 57715) which includes DNA segments inserted into the Hind III site of phage charon 21A. Out of 186 randomly picked up plaques that do not hybridize to the human female genomic DNA, 103 had inserts of approximately 4Kb fragments. Among them, nine Y-specific DNA fragments were isolated. Of the nine clones, two (Y-25 and 80) revealed unique sequences and the other seven clones (Y-48, 50, 102, 111, 146, 148 and 153) revealed Y-specific sequences in addition to X and or autosomal sequences. Nucleotide sequencing of the Y-80 clone was further determined. The clone was 4.6 kilobase pairs long. Oligonucleotides flanking a PSTI-EcoRI fragment of the Y-80 clone was used for polymerase chain reaction. The male specificity of the PSTI-EcoRI fragment was proven through its amplification by polymerase chain reaction using male and female genomic DNA as a template. The polymerase chain reaction proceeded when the male genomic DNA was used as a template, whereas it did not when the female genomic DNA was used.

C 43

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF X CHROMOSOME AMONG JAPANESE POPULATION.
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Restriction fragment length polymorphisms were studied among the Japanese population using 25 polymorphic DNA probes on the X chromosome. The allelic frequencies were quite different ($p < 0.01$) from those for Caucasians for 8 probes (p114.12, St14-1 (*Taq*I), 36B-2, MN12, dic56, pOTC (*Msp*I), pTAK8B and pXG-16 (*Hind*III)). No polymorphisms were observed for 5 probes (p114.12 (*Hind*III), pG95α1-7dIII/RI, pXG-16 (*Taq*I), p8 (*Taq*I) and pXG-17). Our results suggest that 12 DNA probes (p482.6a, p43-15, 52A, St14-1, p114.12 (*Bcl*I), 36B-2, MN12, pPA4B, pOTC (*Msp*I), cPX203, p58-1 and pHPGK-7e) are useful (PIC > 0.42) for linkage analyses of X-linked diseases in Japan.
We have also done the carrier detection in a family of adrenoleukodystrophy (ALD) with St14-1 probe, which had been shown to have a tight linkage to ALD gene.

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A DIAGNOSTIC STUDY OF HYDATIDIFORM MOLE USING POLYMERASE CHAIN REACTION (PCR) TARGETING THE APOB VNTR.Ryuichi FUKUYAMA, Kosuke SAKAI, Jun KUDOH and Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo), Mizuho TAKADA and Syozou TAMURA (Dept. Gynecol. Keio Univ. Sch. Med., Tokyo)

The 3' flanking region of the apoB gene includes a VNTR which often differs in repeating number in each allelic chromosome. We applied the polymerase chain reaction (PCR) to target this VNTR using a small amount of clinical material in order to analyze and diagnose hydatidiform moles. We first determined the size distribution of Japanese alleles using DNAs obtained from bloods and normal tissues. One band or two bands were detected in each individual and size varied from 600bp to 900bp. The pattern of restriction enzyme digestion with HinfI and SspI showed that these bands were targeted sequences. Heterozygosity index was 60.6%. Using five samples of mole DNAs, only one band was consistently detected and its size was equal to one of the paternal alleles, indicating that the moles examined were all parthenogenetic. The method is rapid and sensitive for diagnosis of hydatidiform mole and will be useful for other sociomedical investigations.

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CHROMOSOMAL ASSIGNMENT OF THE HUMAN AORTIC SMOOTH MUSCLE ACTIN GENE.
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The human aortic smooth muscle actin gene has been cloned, and its unique sequence (the 2.7 kb *Eco*RI-*Hind*III fragment containing the 1st exon) was used as the hybridization probe for DNAs of 18 human-rodent cell hybrids. The hybridization data showed that the gene is located on human chromosome 10, which is different from other actin genes so far examined. By *in situ* hybridization the gene was regionally mapped to the long arm (q22-q24) of the chromosome.
A *Taq*I RFLP was detected by the 2 kb *Eco*RI fragment (pASMDe), which was derived from the 3' flanking region of the gene. The allelic frequency was determined by analyzing DNAs of 31 unrelated (including 5 Caucasians) individuals, to be 81:19 (8.5 kb allele:5 kb + 3.5 kb allele). This RFLP was also detected in the Caucasians.

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HUMAN GENE MAPPING UTILIZING FLOW-SORTED CHROMOSOMES AND A HYBRID CELL PANEL. Jun KUDOH, Shinsei MINOSHIMA, Ryuichi FUKUYAMA, Masahiko MAEKAWA, Kosuke SAKAI, Masamichi HIRAI and Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo)

We have identified the chromosomal localization of several human genes by a combination of spot-blot hybridization to flow-sorted human chromosomes and Southern blot analysis of DNAs from a panel of human-mouse cell hybrid clones.

Using these methods, we were able to localize nine human genes to particular human chromosomes: 80K-H protein (G19P1), a substrate for protein kinase C, to chromosome 19; calpain large subunit 1 (CAPN1), large subunit 2 (CAPN2), large subunit 3 (CAPN3) and small subunit (CAPN4) to chromosomes 11, 1, 15 and 19, respectively; heart-skeletal muscle ADP/ATP translocator (ANT1) to chromosome 4; mitochondrial ATP synthase β subunit (ATPSB) to chromosome 12 (12p13-qter) and two ATPSB-related genes (ATPSBL1 and ATPSBL2) to chromosomes 2 and 17, respectively.

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PHYSICAL MAPPING OF HUMAN CHROMOSOMES 21 AND 22. Nobuyoshi SHIMIZU, Shinsei MINOSHIMA, Jun KUDOH, Kazuhiko KAWASAKI, Ryuichi FUKUYAMA and Masahiko MAEKAWA (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo)

We have been involved in the physical mapping of human chromosomes 21 and 22 using a "top-down strategy" in order to facilitate the isolation of the genes causing genetic disorders such as familial Alzheimer's disease and acoustic neurofibromatosis. We have developed a flow-sorting method to isolate a large number of these chromosomes in an intact form that is suitable for analyzing and obtaining giant DNA fragments. One of the two homologous chromosomes of 21 and 22 were sorted from B-lymphoblastoid cells which have translocation chromosome t(10;21)(p11.2;q22.3) and t(11;22)(q23;q11), respectively. DNAs from approximately 500,000 single homologs of chromosomes 21 and 22 were analyzed by pulsed field gel electrophoresis after digesting with the rare cutting restriction endonuclease NotI. Southern blot hybridization using a human Alu DNA probe revealed at least 25 and 30 giant DNA fragments (100 kb to 2.5 Mb in size) for chromosomes 21 and 22, respectively. Accumulative sizes of these Alu^+ NotI fragments were over 33% and 36% of the entire DNA sizes of chromosomes 21 and 22. We have cloned the NotI fragments into yeast YAC55S2 vectors and constructed a chromosome-specific NotI linking library. Characterization of these clones is currently in progress.

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Localization of two different genes for human pregnancy-specific β1-glycoprotein(PSβG) to chromosome 19 at band q13.2

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Pregnancy-specific β1-glycoprotein, which belongs to a CEA gene family, is a heterogeneous product of synsytiotrophoblast and found in neoplasms of various origines. Recently, two different genomic fragments for the different PSβGs were isolated, and designated CGM35 and PSβG HL, respectively (Oikawa et al, Biochem Biophys Res Commun, 1988, 1989). We attempted to localized the two PSβG genes on a human chromoosme by in situ hybridization. The hybridization analyses on the hybrid cell DNA and sorted chromosomes allowed the assignements of both CGM35 and PSβG HL to chromosome 19; the in situ hybridization experiments localized both the genes to band q13.2.

誌上発表

遺伝相談と出生前診断 — そのかかわり：又吉國雄、井槌慎一郎、吉田啓治、秋谷清（東京医科大学産婦人科）CORRELATION BETWEEN THE GENETIC COUNSELING AND PRENATAL DIAGNOIS:Kunio MATAYOSHI, Shinichiro IZUCHI, Keiji YOSHIDA and Kiyoshi AKIYA(Dept. of Obstet. and Gynecol., Tokyo Medical College, Tokyo)

遺伝相談の中で出生前診断は年々多くなって来ている。その背景には、診断技術の向上や、社会的少産少育傾向から、両親にもより良い資質を備えた子供を欲する願望が強くなっていることがあげられる。実際に産科医の側にも、それに応えるように一般診療の場で出生前診断が行われ、異常とわかれば中絶するということが、行われていなくもない。このような点から、出生前診断と遺伝相談とのかかわりを考えてみたい。

遺伝相談とは、clientとcounselorとの対話過程であり、根底に遺伝的危険率があることは言うまでもない。しかし実際には危険率確率論だけでは処し得ないことも多く、危険率が小さくてもclientが不安を拭い得ず中絶の転帰をとることもあり、逆に危険率が高いことで挙児をあきらめることもある。その時、出生前診断可能な疾患なら、counselorはclientにその方法を教える必要があり、その時、羊水穿刺、CVSなどは大きな手段となる。そこに出生前診断と遺伝相談との大きな接点が見出される。換言すれば、出生前診断は決してそれ自体独立して行われるべきものではなく、あくまでも遺伝相談の過程を経て行われるべきものであり、一診断手技と同一視すべきものでないことを明確にする必要があろう。