日本人類遺伝学会 第33回大会一般講演要旨

Abstracts of General Contribution, the 33rd Annual Meeting of the Japan Society of Human Genetics

A 1

A SPORADIC CASE OF THE WAARDENBURG SYNDROME WITH de novo CHROMOSOMAL ABNORMALITY AT 2q35. Satoshi ISHIKIRIYAMA, Yoshitaka SIBUYA, Hidehumi TONOKI (Dept. Pediat. Hokkaido Univ.) and Shinki CHIN (Dept. Opht. Hokkaido Univ.)

A boy of one year and eight months suffered from the Waardenburg syndrome type I. Heterochromia irides and vitiligo/hyperpigmentation on the right forearm were noted at his birth. Auditory brainstem response revealed sensorineural deaffness which was severe and bilateral when he was one year and three months old. We noticed mild synophrys, wide nasal root and lateral displacement of inner canthi on the physical examination. His development and growth were not retarded, except for language. None of his relatives shared these findings. On cytogenetical analysis with GTG banding, there was a constitutional abnormality at 2q35. The abnormality looked like an inversion from 2q35 to 2qter. The karyotypes of his parents were quite normal.

The Waardenburg syndrome expresses wide variety, and is subdivided into three categories: type I, type II, and type III. So-called continuous gene syndrome may include the Waardenburg syndrome. The coexistence of the inversion at 2q35 with the Waardenburg syndrome suggests that several genes related to the Waardenburg syndrome might be located around 2q35.

A 2

A CASE REPORT OF SEVERE CEREBELLAR ATAXIA FROM A de novo TRANSLOCATION, t(X;6)(q28;q14.2). Masako SAITOH[1], Michiko OKADA[1], Satoru UEDA[2], Kura KUBOTA[1] and Yoshiko NOMURA[1] ([1]Chromosome Lab., Shiseikai Dai-Ni Hosp., Tokyo; [2]Dept. Pediat., Tokyo Women's Med. Coll. Daini Hosp., Tokyo)

A 10-year-old girl was diagnosed as notanencephalia, hypoplasia of pons~mesencephalon and expansion of ventriculi. She showed developmental delay noticed at age 9 months, severe mental retardation, incoordination, abasia, hypotonia, choreic movement of the upper part of the body, epilepsy since age 2 years, and pubic hair and mammae development at age 7 years. Her parents and 4-year-old brother were healthy. Karyotyping of the patient by EB high-resolution G-band (GTG) and R-band (RBG) revealed a reciprocal translocation; 46,X,t(X;6)(q28;q14.2), while her mother had normal karyotype. The replication study indicated that the normal X was late replicating in 100% of the observed cells, and the der(X) was not late replicating along with the translocated #6 long arm. The damaged features (cerebellar ataxia and precocious puberty) appeared to be associated with the minor karyotypic imbalance at the breakpoints of chromosome X and 6.

A 3

A DE NOVO CASE OF 3Q2 TRISOMY WITH INS(8;3)-APPLICATION OF PERSONAL COMPUTER DIAGNOSTIC SYSTEM FOR ANALYSES OF SMALL DE NOVO EXTRA CHROMOSOMAL SEGMENTS-

Kenji NARITOMI, Yoshinori IZUMIKAWA, Chuken MIYAGI and Kiyotake Hirayama (Dep. Pediatr., Univ. the Ryukyus, Okinawa)

It is often difficult to identify the nature of a small de novo extra chromosomal segment. We tried to analyse a 1-year-old dysmorphed girl showing 8p+ using a personal computer system and several banding techniques in prometaphase cells.

(Personal computer system) Total 247 clinical findings were selected in about 90 chromosomal syndromes respectively. The system was designed to select maximum 10 syndromes within a few seconds using 'basic', if several combinations of findings were input (max. 10).

The patient had about 30 clinical findings. The computer selected two possible syndromes; 3q2 trisomy and 10q2 trisomy (monosomies were neglected). Comparison of chromosomes 3 and 10 by high resolution GTG, QFQ and RBG banding techniques revealed a karyotype; 46,XX,-8,+der(8), dir ins(8;3)(p21.2;q26.1 or 26.2q28 or 29). Dose effect of somatostatin, which is coded at 3q28, confirmed the result (patient 23 for control 1.0-12.2 pg/ml).

It was concluded that personal computer system would help to analyse malformed patients with a marker chromosome.

A 4

A CASE OF A 2p23 TRISOMY DERIVED FROM THE MATERNAL 2/22 TRANSLOCATION.

Hisashi TSUJII, Naoko YOSHIDA, Yasuo DOI, Akihiko KINUGASA, Tadashi SAWADA (Dept. Pediat. Div. Perinat. Unit.), Johji INAZAWA, and Tatsuo ABE (Dept. Hygiene. Kyoto Pref. Univ. Med., Kyoto)

We report here the 12th case of 2p23 trisomy with a karyotype of 47,XY, inv(9)(p11.2q21.2)pat,+der(22),t(2;22)(p23;q11)mat. The proband was male and born at 41 weeks' gestation by vacuum extraction, whose parentsare not consanguinous. Their ages are 26 (primipara mother) and 30 (father). He was not doing well and admitted to our hospital. The physical examination revealed cryptorchidism, a peculiar face with high prominentforehead, large and low set ears, wide flat nasal bridge, pug nose and micrognathia, and long fingers and toes with grasping thumb, widely spaced toes and cubitus valgus. These findings are common to the other reported cases of 2p23 trisomies. However, our case showed the left hydronephrosis, the vesico-urethral reflux and the bilateral optic and chorio-retinal atrophies, which are rare findings in other reported cases.

A 5

INTERSTITIAL DELETION OF THE PROXIMAL LONG ARM OF CHROMOSOME 4 ASSOCIATED WITH FATHER-CHILD INCOMPATIBILITY WITHIN THE Gc-SYSTEM, PROBABLE REDUCED GENE DOSAGE EFFECT AND PARTIAL PIEBALD TRAIT.

Yoshifumi YAMAMOTO(Dept. Pediatr. Jichi Med. Sch., Tochigi), Hiroshi NISHIMOTO(Dept. Neurosurg. Saitama Child. Med. Cen., Saitama) and Shigenori IKEMOTO(Dept. Leg. Med. and Lab. Hum. Biol., Jichi Med. Sch., Tochigi)

Gc-system typing by isoelectric focusing polyacrylamide gel electrophoresis and quantitative assays were carried out on a patient with a karyotype of 46,XY,del(4)(q12q21.1) and on his parents with normal chromosomes. Although a father-child incompatibility within the Gc-system suggested that its locus is on the segment 4q12-13, the serum concentration of vitamin D binding protein(Gc-system) in the patient and his father were only about half of those of his mother and the controls. In our case, the possibility of interference of a silent allele to the father-child incompatibility could not be completely excluded.

Although the extent of distribution of congenital leukodermia is very limited in our patient, associated skin changes appeared to be an expression of a partial piebald trait. To clarify the genetic distance between the Gc-system and the piebald trait, further studies including quantitative assays of the Gc-system in patients and their parents are needed in appropriate clinical cases.

A 6

A CASE REPORT OF TERMINAL DELETION OF THE LONG ARM OF CHROMOSOME 16 AND PRESUMPTION TO GENE LOCUS OF HAPTOGLOBIN.

Fumio TAKADA, Shouji YAMANAKA, Kiyoshi IMAIZUMI, Yoshikazu KUROKI (Dept. Med.Genet., Kanagawa Cildren's Medical Center, Kanagawa)

A 10 month old boy with 16q terminal deletion was presented. He was born as the third child to healthy and unrelated parents after an uneventful course of pregnancy and normal delivery. The karyotype was 46,XY,del(16)(q22.1). This is the first report of the terminal deletion of 16q in Japan. Clinical features included growth retardation, mental retardation, hyperhidrosis, frontal bossing, dileted scalp veins, anti-mongoloid slant, hypertelorism, persistent papillary membrane, broad and flat nasal bridge, low-set and dysmorphic ears, velopharyngeal insufficiency, micrognathia, short neck, wide set nipples, diastasis recti, small hands and feet and right renal hypoplasia. These features were compatible with those of 16q- syndromes in the literature. Haptoglobin gene has been assigned to 16q22. The locos in the present case was intact by haptoglobin haprotype analysis. Thus SRO of Haptoglobin gene was suggested to be 16q22.1.

A 7

REGIONAL MAPPING OF THE PARATHYROID HORMONE AND THE CALCITONIN GENES TO 11p15.3-11p15.1 BY CYTOGENETIC AND MOLECULAR STUDIES.
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The loci for the human parathyroid hormone gene (PTH) and the calcitonin gene (CALCA) were assigned to 11p15.3-11p15.1 by a cytogenetic and molecular genetic study performed on a patient with the Beckwith-Wiedemann syndrome accompanied with a chromosomal abnormality [46,XX,-14,+der(14),t(11;14)(p15.3;q32.2) pat]. The gene doses of PTH and CALCA, and several other genes (HRAS, INS, IGF2, D11S12) that are all localized in 11p15 were tested with densitometry on autoradiograms of Southern blot hybridization. The copy numbers of PTH and CALCA genes were both two in the patient, whereas those of other genes tested were all three. The two copy density for PTH was confirmed by RFLP analysis on the patient and her parents. Our data is inconsistent with the previous map that both PTH and CALCA were assigned to 11pter-11p15.4.

A 8

MOLECULAR-GENETIC STUDY OF PRADER-WILLI (PWS), ANGELMAN (AS), WILLIAMS (WS) AND BARDET-BIEDL (BBS) SYNDROMES.
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DNAs from a total of 26 cases [18 PWS with different karyotypes, 6 AS, 1 WS and 1 BBS patients] were analyzed with Southern hybridization using two genomic sequences, pML34 and pTD3-21, as probes in order to know the copy number of the sequences in their genomes, since the 15q11-12 deletion has been reported in these syndromes and/or some syndromes share with several specific manifestations. Of 15 PWS cases with del(15)(q11.1;q12), 13 had a molecular deletion. Two sporadic AS cases also had a molecular deletion. However, two PWS cases with del(15q), two PWS cases with t(15;15), 1 PWS case with inv dup(15q), 4 AS cases (2 sporadic and 2 familial cases), and one each case of WS and BBS all had two copies of both the pML34 and pTD3-21 sequences in their genomes. These findings indicate that the molecular change in both PWS and AS is not always parallel to its chromosomal change. Another conclusion is that the putative gene(s) for AS may locate closely to or overlap the PWS gene(s), i.e. both syndromes are the contiguous gene syndromes.

A 9

CYTOGENETIC and FAMILY STUDIES of THE ANGELMAN SYNDROME.
Kiyoshi IMAIZUMI, Fumio TAKADA, Yoshikazu KUROKI (Dept. Genet. Kanagawa Child. Med. Cent.), Kenji NARITOMI (Dept. Pediat. Ryukyu Univ. Okinawa)

The Angelman syndrome was first described by Angelman (1965) with severe mental retardation, absent speech, siezure disorder, easily provoked paroxysms of laughter and puppet-like jerky gait. Fifty two cases have been reported. Most cases were sporadic, while three reports of sibs and one case of monozygotic twins were reported. Segregation analysis including our new patients revealed that the segregation ratio would be situated between 0.066 to 0.123. Using high resolution banding method, cytogenetic analyses were performed in our 6 patients, 4 were sporadic cases and two were sibs. Interstitial deletion of band q11.2-q12 in one chromosome 15 were detected in all sporadic cases, while in affected sisters no deletion could be recognized. As these deletion were similar in many cases of the Prader-Willi syndrome, we suggested that different molecular abnormalities of specific segments revealed the clinical heterogeneity between these two syndromes.

A 10

CLINICAL HETEROGENEITY ASSOCIATED WITH DELETIONS IN THE LONG ARM OF CHROMOSOME 15.
Yoshikazu KUROKI, Kiyoshi IMAIZUMI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama) and Norio NIIKAWA (Dep. Genet., Nagasaki Univ. Schl. Med., Nagasaki)

Karyotype-phenotype correlation is a widely accepted concept in clinical cytogenetics. Recently, clinical heterogeneity associated with deletions in the long arm of chromosome 15 has been raised several times. Although most cases were associated with the Prader-Willi syndrome (PWS), several non-PWS cases were reported. These included the Angelman syndrome, the Williams syndrome and unkown MCA/MR cases. In the present study, we delineated clinical features of 26 cases with the proximal 15q deletions, examined in the Kanagawa Children's Medical Center during the period 1983 - 1987. Clinical evaluation revealed that 23 cases suffered from the PWS and the rest 3 from the Angelman syndrome. But a boy with the Williams syndrome showed no 15q deletion. Gene deletions were confirmed in most cases with the PWS by the use of DNA probes isolated from 15q11.2. Using the same probes, however, gene deletions were confirmed only in 2 cases out of 6 with the Angelman syndrome. In conclusion, the heterogeneity associated with the same 15q deletion was confirmed in both clinical and molecular level.

A 11

CLINICAL, CYTOGENETIC AND EPIDEMIOLOGICAL STUDIES OF 62 KABUKI MAKE-UP (KMS) PATIENTS. Norio NIIKAWA*(Dept. Hum. Genet., Nagasaki Univ., Nagasaki), Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Hosp., Yokohama), Tadashi KAJII (Dept. Pediat., Yamaguchi Univ., Ube)

Sixty-two KMS patients were studied to delineate its phenotypic spectrum and to learn about the cause. Five cardinal features observed included (1)a peculiar face(100%) with eversion of the lower eyelid, a depressed nasal tip and prominent ears;(2)skeletal anomalies(92%), including short fingers V and a deformed spinal column; (3)dermatoglyphic abnormalities(93%), including increased UL and hypothenar loop patterns, absence of triradii c and d, and presence of fingertip pads; (4)mild to moderate mental retardation(92%); (5)postnatal growth deficiency(83%). The core of the spectrum is narrow and clearly defined. Important inconsistent anomalies were early breast development in females(23%) and CHD(31%). Of 62 patients, 58 were Japanese, indicating that KMS is fairly common in Japan. Its prevalence in Japanese is 1/32,000. All the KMS cases were sporadic. the sex ratio was even, the consanguinity rate among the parents was not high, and no incriminated agent was found that was taken by the mothers during pregnancy. Three of 62 cases had a Y chromosome abnormality involving a possible breakpoint (Yp11.2), not inconsistent with an autosomal or pseudoautosomal dominant disorder in which every patient represent a fresh mutation. The mutation rate was calculated at 15.5×10^{-6}.

A 12

FREQUENCIES OF INV(9) CHROMOSOME IN NORMAL AND VARIOUS PATIENT POPULATIONS OF 3,000 JAPANESE. Kiyomi YAMADA (Div. Genetics, Clin Res Inst, Nat Med Cent Hosp, Tokyo)

Using a C-banding method by DA-DAPI double staining, the frequencies of individuals carring a pericentric inversion of No. 9 chromosome have been studied in a total of 3,163 individuals of 6 groups; normal healthy volunteers (NR), Down syndrome patients (DW), Klinefelter patients (KF), azoospermic male patients (AZ), couples with repeated abortion history (HA), and spontaneously aborted fetuses (SA).

Population frequencies were found to be 0.015 in the NR group (n=1,122), 0.013 in the DW group (n=922), 0.018 in the KF group (n=217), 0.011 in the AZ group (n=186), 0.041 in the HA group (n=410), and 0.033 in the SA group (n=150). In the above calculation, couples (n=18) and aborted fetuses(n=120) with abnormal karyotypes were excluded. Sex difference due to the slight preponderance of female carriers was recognized in the NR and DW groups, and aborted fetuses having an inv (9) in addition to other chromosome abnormalities were extremely rare.

The present data suggest that an inv(9) chromosome seems to have a genetic load toward the reduction of viability in human ontogenic development and consequently the carriers are prone to the fetal loss in reproduction.

A 13

ANALYSES OF BREAKPOINTS OF RECIPROCAL TRANSLOCATION CHROMOSOMES WITH BALANCED CONDITIONS IN MAN. Hidetsune OISHI (Dept. Genet., Inst. Develop. Res., Aichi Prefect. Colony, Kasugai), Tsutomu YAMANAKA (Cent. Hosp., Aichi Prefect. Colony, Kasugai), Kaoru SUZUMORI (Dept. Obs. Gynec., Nagoya City Univ., Nagoya) and Ken HAYASHI (Dept. Obs. Gynec., Kyoto Univ., Kyoto)

The frequencies of autosomal rearrangements with reciprocal translocation were estimated from our records and published reports. By the pedigree analyses the balanced conditions of chromosomal rearrangement for two or more generations were also ascertained in every family of them. In 266 families examined, male and female probands were 132 each and no record for sex of probands in 2 cases, while their fathers and mothers with the balanced condition were 78 and 188, respectively.

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. There is an excess of breakpoints on chromosomes 5p, 9p, 18q, 21q and 22q and a dearth on chromosomes 1p, 2, 3q, 5q, 6q, 8q, 11p and 19q. This distribution is quite different from that of a sample of reciprocal translocations ascertained for recurrent abortions by Campana et al.(1986), except those on chromosomes 2, 5q, 11p and 22q.

A 14

ORIGIN OF A MINUTE CHROMOSOME SEGMENT TRANSLOCATED TO 9q34.3. Naoki HARADA*, Kyohko ABE (Kyushu Med. Sci., Cytogenet. Dept., Fukuoka), Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki)

A 5-year-old boy with Williams syndrome-like features had fragile site-looking chromosome 9. Observation of this unusual chromosome in 100% of lymphocytes cultured with or without FrdU and absence of triradial figures ruled out fragile sites. NOR- and Cd-bandings revealed silver dots and separated centromere dots, respectively, around the gap of the #9, indicating that a small minute segment with inactivated centromere is translocated to 9q34.3. QFQ-banding revealed a moderately brilliant spot, but DA/DAPI staining showed no fluorescence dot at the 9qter. Heteromorphic marker studies on the patients and parents by QFQ-, RFA-, and AluI-C bandings were then performed to know the transmission of the marker from parent of child and to identify the translocated segment, showing that the satellited chromosome 9 is resulted from de novo t(9;22)(pter-q34.3::q11.1-pter).It was concluded from C-heteromorphisms that the translocation had occurred at the paternal gametogenesis, not inconsistent with the previous hypothesis on the parental origin of de novo structural abnormality. This unusual satellited chromosome has not been known in the literature. If we assume that the patient suffers from Williams syndrome (WS), the locus of a putative WS gene would be at either 22pter-q11.1 or 9q34.3-qter, inconsistent with the previous tentative locus (15q11-12).

A 15

CELL DIVISION KINETICS OF RING CHROMOSOMES. Masae Murakami, Kouji Narahara, Kiyoshi Kikkawa, Hiroshi Nanba, Kei Hiramoto, Hiroshi Kimoto (Dept. Pediatr., Okayama Univ., Okayama) and Ryozou Kasai (Asahigawa Jidoin Hosp., Okayama).

A phenotype of a ring chromosome depends not only on the size of deletion involved but also on the instability of the ring chromosome itself. To reassess the 'instability' cytogenetically, we studied changes in frequencies of various ring forms in consecutive cell cycles in 4 cases with r(3), r(9), r(13) and r(22) and 2 cases with r(X) in mosaicism, using FPG technique. In the 4 cases with autosomal rings, ratios of cells with a monocentric ring decreased and those with ring variants increased with advancing cell cycle. Frequencies of cells with monosomy, two monocentric rings and a dicentric ring, however, were constant irrespective of cell cycle. In the 2 cases with r(X), on the other hand, ratios of cells with 45,XO decreased and those with a monocentric ring and its variants increased with advancing cell cycle. These results suggested that the sum total of ratios of cells with monosomy, two monocentric rings and a dicentric ring may best represent an in vivo instability of a ring chromosome in cases with autosomal rings. The discordance in cell division kinetics between autosomal and X chromosomal rings can be explained by the different viability of secondary aneuploid cells in a culture system.

A 16

A CASE WITH KLINEFELTER SYNDROME HAVING BEEN DIAGNOSED AS "SPAETSCHIZOPHRENIE". Akio ASAKA, Takashi TAMIYA (Dept. Mental Health, Tokyo University, Tokyo and Tamiya Mental Hospital, Nagaoka)

The patient is a 58-year-old married male. No positive family history as to neuropsychiatric disorders was found. He suffered from congenital Glory Sun syndrome, and gradually lost his eyesight. At the age of 51, his eyesight was remarkably decreased, and he had to stop his carpenter-like job. At that time he experienced some troubles with his neighbourhood about the clearance of heavy sknowfall. By that moment, he suddenly become mentally disturbed. Auditory and visual hallucination, dselusion of reference and jealousy, monologue, excitement, violent behaviours against his wife and so on were observed. After the first admission at the age of 52, he repeated it several times. During his 3rd admission, it was noticed that he had no axillary hair and his voice was high-pitched and besides he had no child. Laboratory examination revealed that he had the karyotype 47,XXY and high gonadotropin. He showed no personality deterioration, disturbed contact, or flattening of affect usually seen in typical schizophrenia. In 1968 we have already reported the characteristic features of psychotic symptoms observed in Klinefelter syndrome. They shows usually periodic affective sways overlapped by schizophrenia-like symptoms.

A 17

CYTOGENETIC POPULATION STUDIES OF CYNOMOLGUS MONKEYS.
Momoki Hirai (Dept. Anthropol., Tokyo Univ., Tokyo), Keiji Terao, Fumiaki Cho and Shigeo Honjo (Tsukuba Primate Center, NIH, Tsukuba)

Chromosomal abnormalities have not often been reported in primate species other than man. A cytogenetic population survey was carried out among a total of 297 cynomolgus monkeys (Macaca fascicularis, 2n=42) kept at the Tsukuba Primate Center for Medical Science in an attempt to detect chromosomal abnormalities. One male was found to show a mosaic chromosome constitution involving the Y chromosome, 42,XY/43,XYY. The frequencies of cells having an abnormal karyotype with an additional Y chromosome in cultured lymphocytes, kidney cells and spermatogonia were 14%, 5.4% and 4.3%, respectively. No effect of this chromosomal deviation on the phenotype of the subject had been detected. One female showed a possible paracentric inversion at the proximal region involving the centromeric heterochromatin of the long arm. These results, although derived from a small number of individuals, suggest that further cytogenetic population studies in non-human primates might lead to the discovery of the more cases of chromosomal abnormality and perhaps provide information on the causes and consequences of such chromosomal imbalance in man.

A 18

MOLECULAR CLONING OF A DNA REPAIR GENE THAT COMPLEMENTS THE DEFECT OF GROUP A XERODERMA PIGMENTOSUM.
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Xeroderma pigmentosum(XP) is an autosomal recessive human disease, characterized by skin cancer-proneness. Cells from XP patients are hypersensitive to killing by UV-light because of their defect in repair of UV-light induced DNA damage. For isolation of the gene responsible for XP complementation group A, the pSV2gpt and genomic DNA from mouse embryo were co-transfected into XP2OSSV group A XP cells. Two primary UV-resistant XP transfectants were isolated from about 1.6×10^5 pSV2gpt transformed XP colonies. The pSV2gpt and genomic DNA from the primary transfectants were again co-transfected into XP2OSSV cells and a secondary UV-resistant XP transfectant was obtained by screening about 4.8×10^5 pSV2gpt transformed XP colonies. The secondary transfectant retained fewer mouse repetitive sequences. A mouse gene that complements the defect of XP2OSSV cells was cloned into EMBL3 phage vectors from the secondary transfectant. Transfections of the cloned DNA also conferred UV-resistance on another group A XP cell line, but not on group C, D, F or G XP cell lines, suggesting that the cloned DNA repair gene is specific for group A XP and may be the mouse homologue of the group A XP human gene.

A 19

ISOLATION AND CHARACTERIZATION OF APHIDICOLIN-RESISTANT HUMAN CELL LINES. Kouzin KAMINO, Jun NAKURA, Yuji TAKEMOTO, Tetsuro MIKI, Toshio OGIHARA, Yuichi KUMAHARA (Dept. Med.Geriat., Osaka Univ., Osaka) and Kiyoji TANAKA (Inst. Mol. Cell. Biol., Osaka Univ., Osaka)

We established four aphidicolin(aph)-resistant human cell lines isolated from mutagenized HeLa cells with ethylmethanesulfonate and UV-light. The cell cycles of the mutants were 20 to 50% longer than that of HeLaS3 cell and all mutants showed UV-resistency. It has been reported that aph-resistency is induced by the alteration of enzyme activity of DNA polymerase α , ribonucleotide reductase or thymidylate synthetase. The direct counts of growth rate revealed that all mutants showed the resistency for hydroxyurea that inhibits ribonucleotide reductase. But both Southen and Northern analysis using M2 subunit cDNA of ribonucleotide reductase (gifted by Dr. Lars Thelander) demonstrated no gross alteration in the mutants. And these mutants also showed the resistency for 5-fluorodeoxyuridine that is an inhibitor of thymidylate synthetase, and so it was suggested that these mutants had an alteration of the regulation of dNTP pools. And the fibroblasts derived from patients of Cockeyne syndrome, one of progeroid syndrome, were shown to be sensitive for aph as well as for UV. Therefore, the altered genes of these mutants were assumed to be related to the aging.

A 20

ESTABLISHMENT OF A NIH3T3 DERIVED CELL LINE WHICH CAN BE INDUCED EXPRESSION OF INTEGRATED HPV16 E6, E7 REGION BY GLUCOCORTICOID HORMONE. Hidenori KATO, Takafumi FUJINO, Norio Wake, and Seiichiro FUJIMOTO (Dept. Obstet. Gynecol., Hokkaido Univ., Sapporo) Toshiharu YAMASHITA, and Kei FUJINAGA (Dept. Mol. Biology, Cancer Res. Inst., Sapporo Med. College, Sapporo)

The association of human papilloma virus type 16 (HPV 16) with the development of malignancy could be ellucidated by many experimental evidences. The E6 and E7 regions are assumed to be the most important although the definite role of these genes in the transformation process yet remains to be clarified. Thus, we have established the experimental system to analyse the functions of these regions.

The plasmid construct consisted of the HPV16 Hae III 2.1Kb fragment involving the E6 and E7 regions ligated downstream with LTR of mouse mammary tumor virus. The plasmid and the neo gene were cotransfected into the NIH 3T3 cells. The selection with the G418 resulted in the production of many clones in which the Hae III 2.1Kb fragment was integrated successfully. The regulation of E6 and E7 expression by glucocorticoid (DXM) could be recognized by Northern blot analysis in one clone. This clone was not tumorigenic but the saturation density seemed to be increased with the hormone, compared to the one of original cells, suggesting the availability of the clone for investigations of E6 and E7 functions during the transformation process.

A 21

A-TYPED COCKAYNE SYNDROME : A CASE REPORT OF TWO NEW SIBLINGS.

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Feasibility of DNA duplication synthesis and RNA synthesis after UV-exposure or a defect in DNA ligase are suggested as a causative defficiency of Cockayne syndrome.

CASE 1 : The first boy of healthy and non-consangeneous parents was delivered after a previous spontaneous abortion. He had mental and physical retardation, precocious senile appearance, hypersensitivity to UV-exposure, calcification in basal ganglions of the brain, cataracta, optic atrophy, retinal degeneration and progressive contracture of joints. He had a stroke at 16 year old. And one year later he died of renal insufficiency. Autopsy revealed renal atrophy, fatty liver and Meckel's diverticulm.

CASE 2 : Same parents had this third boy after one healthy male and two artificial abortions. His mother took him to us because of resemblance to the case 1.

Cultured skin fibroblasts of both cases have high UV-sensitivity, while unscheduled DNA synthesis is within normal levels. Complementation tests showed both to be A-typed for deficits of their RNA synthesis after UV-exposure.

A 22

CHROMOSOME ASSGINMENT OF THE PCNA GENE FOR HUMAN PROLIFERATING CELL NUCLEAR ANTIGEN.

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The proliferating cell nuclear antigen (PCNA, also called cycline) is a protein synthesized only during the S phase of the cell cycle, and identical with a DNA polymerase-δ acessory protein. Recent studies suggest that PCNA stimulates DNA replication and increases the processivity of DNA polymerase-δ , indicating that PCNA is a protein implicated in cell cycle control and chromosomal DNA replication. Using a cDNA probe to the PCNA we localized the PCNA gene to a human chromosome by in situ hybridization method. Silver grains were distributed on chromosomes 2, 3, 8, 12, 14, and 15 with a large accumulation on the long arm of chromosome 2 at bands 2q31→q35. A significant distribution of the grains was on 2q33.

A 23

CHROMOSOMAL ASSIGNMENT OF THE ALCOHOL DEHYDROGENASE CLUSTER LOCUS TO HUMAN CHROMOSOME 4q21-23 BY IN SITU HYBRIDIZATION.

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Human class I alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase, EC 1.1.1.1,Abbreviation ADH) is the major enzyme involved in ethanol oxidation. Three highly homologous genes govern the synthesis of three types of subunits that form several ADH isozymes. The loci for class I ADH was previously assigned to q21-25 of chromosome 4 by somatic cell hybridization techniques. Analysis of grain positions by in situ hybridization of chromosomes indicated that the ADH cluster locus is on 4q21-23, probably on 4q22.

A 24

COMPARATIVE ANALYSIS OF TWO METHODS OF IN SITU HYBRIDIZATION IN GENE MAPPING.

Kaoru TAKEDA, Hiroshi NAKAI, Yoshitsugu YAMAMOTO, Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai)

INTRODUCTION: Two methods of in situ hybridization were compared to know differences between Q-stained metaphase and G-banded prometaphase preparations for gene mapping. MATERIALS AND METHODS: Chromosomes were prepared from human lymphocytes of a normal male. Using cDNAs of insulin-like growth factors-1 & -2, ^{3}H-dATP, ^{3}H-dTTP, ^{3}H-dCTP and non-radioactive dGTP, we labeled probes looking for 1 - 4 x 10^7 cpm/mcg of DNA by nick translation. After hybridization with labeled probes, metaphases were stained by Quinacrine mustard, while prometaphases were G-banded by Hoechst 33258, UV-exposure and Giemsa. RESULTS: In Q-stained metaphases, chromosomes were short and bands were not so clear and faded gradually. On the other hand, G-banded prometaphases had clearer bands on longer chromosomes and these bands did not fade despite of the long-term storage. DISCUSSION: Although the technique has some difficulties, in situ hybridization using G-banded prometaphases is easier for designation of bands on which silver grains locate. Therefore this method is more adequate to finer gene mapping. Gene loci of IGF1 and IGF2 are assigned to 12q22-q24.1 and 11p15 using Q-stained metaphases and to 12q22 and 11p15 by G-banded prometaphase method respectively.

A 25

pSV2neo および pSV2gptマーカーをもつ単一染色体ライブラリーの作製とアイソザイムによる確認. 清水素行[1,2]・森田裕之[1]・山田秀人[1]・児井 稔[1]・篠田雅人[2]・押村光雄[1]（[1]神奈川がんセ・研・細胞遺伝，[2]星薬大・生化学）. CONSTRUCTION AND ISOZYME ANALYSES OF HUMAN MONO-CHROMOSOMAL LIBRARIES WITH pSV2neo AND pSV2gpt MARKERS. Motoyuki SHIMIZU[1,2], Hiroyuki MORITA[1], Hideto YAMADA[1], Minoru KOI[1], Masato SHINODA[2] and Mitsuo OSHIMURA[1]([1]Lab.Cytogenet.Kanagawa Cancer Center Res.Inst., Yokohama; [2]Dept. Biochem. Hoshi Univ., Tokyo)

HGPRT locus を含むX染色体の一部が常染色体に転座した染色体をもつヒトGM細胞，あるいは優性遺伝子マーカーである pSV2neo および pSV2gpt をカルシウム沈殿法により正常ヒト線維芽細胞にトランスフェクションし，形質転換したヒト細胞とマウスA9細胞との雑種細胞を作製した．その雑種細胞から調製した微小核細胞を媒介とする微小核融合法をマウスA9細胞に適用し，３種類の異なった遺伝的選択マーカーを有するヒト染色体を１本だけもつマウスA9細胞のライブラリーを作製した．しかし，これらのライブラリーのヒト染色体はキナクリン・ヘキスト併用染色法による染色体解析のみにより同定されたものであった．そこで，種々のヒト染色体上にその位置の知られる20種のアイソザイムによる解析をそれぞれのライブラリーについて行った．その結果，アイソザイムによる解析結果は染色体解析による結果と一致した．

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ヒト神経芽細胞腫細胞株（SK-N-MC）への正常１番染色体の移入．森田裕之，児井 稔，清水素行，山田秀人，押村光雄（神奈川がんセ・研・細胞遺伝）. INTRODUCTION OF HUMAN CHROMOSOME #1 MARKED WITH pSV2neo VIA MICROCELL FUSION INTO HUMAN NEUROBLASTOMA CELL LINE (SK-N-MC). Hiroyuki MORITA, Minoru KOI, Motoyuki SHIMIZU, Hideto YAMADA and Mitsuo OSHIMURA (Lab.Cytogenet. Kanagawa Cancer Center Res. Inst., Yokohama)

神経芽細胞腫の腫瘍細胞における染色体解析の結果，１番染色体短腕（1p34－pter）における欠失および転座が高頻度に観察されることが明らかにされてきた．一方，ウィルムス腫瘍の発生には１１番染色体における異常を伴うことが知られ，正常由来１１番染色体の移入により造腫瘍性が抑制されることから(Weissman et al.,1987)，ヒト１１番染色体上にウィルムス腫瘍の発生にかかわる遺伝子（群）の存在することが示唆されている．従って，神経芽細胞腫において高頻度にみられる１ｐ染色体欠失より，ヒト１番染色体上にこの腫瘍の発生に関与する遺伝子の存在が予想される．そこで，我々が既に作製した pSV-2neo を優性遺伝子マーカーとするヒト単一染色体ライブラリーより，ヒト１番染色体を１本もつマウス A9 細胞を選び，この細胞よりミクロセルを調製し，SK-N-MC 細胞に融合した．G418 含培地で選択培養後，G418 耐性クローン（ヒト１番染色体の存在が確認されたもの）６個を分離した．さらに，１番染色体の移入された４クローンのヌードマウスにおける造腫瘍性を検索した結果，各々のクローンにおいて造腫瘍性が抑制された．

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CHROMOSOME ABERRATIONS IN LYMPHOID MALIGNANCIES.
Kimio TANAKA, Keiko SAKATANI, Miho TAKECHI, Chiharu SHIGETA, Nobuo OGUMA and Nanao KAMADA(Dept.Hematol., Res. Inst. Nuclear Med. & Biol., Hiroshima Univ., Hiroshima)

Cytogenetic study on 106 cases with lymphoid malignancies was performed to correlate chromosomal findings, histological types and immunophenotypes, and to compare the results with those in USA. Patients were 30 cases of ALL, 34 cases of ATL and 42 cases of malignant lymphoma(ML). Chromosome analysis was done by short-term culture and G-banding method. The results are as follows ; (1) Frequencies of chromosome aberrations in ML(84.4%) and in ATL(86.4%) were much higher than those in ALL(58.6%). (2) B cell-ML had more complex abnormalities. (3) Chromosomes 1, 3, 6, 9 and 14 were frequently involved. Deletion of chromosome 6(6q-) was mostly found in B cell diffuse large cell type. (4) Though breaks in 8q24, 14q32 and 18q21 were seen frequently in USA, these aberrations were less frequently observed in Japan. These differences may come from rarer occurrences of follicular and Burkitt type of lymphomas in Japan. Our results may contribute to the understanding of geo-pathological differences between these countries.

A 28

"JUMPING TRANSLOCATION" OF A TRISOMIC WHOLE q ARM OF CHROMOSOME #1 IN AN ACUTE LYMPHOBLASTIC LEUKEMIA.
Tamiko SHINOHARA (Dept. Hum. Cytogenet., Japan Red Cross Med. Centr., Tokyo), Kazuo DAN, Takeo NOMURA (Dept. Int. Med., Nihon Med. Col., Tokyo) and Akira TONOMURA (Dept. Cytogenet., Tokyo Med. Den. Univ., Tokyo)

The cytogenetic analysis of bone marrow cells from a 59-year-old man with an acute lymphoblastic leukemia (ALL) is reported. He was pointed out an anemia, thrombocytopenia and the presence of many atypical abnormal cells in peripheral blood, and was admitted to the Nihon Medical college Hospital on Sept. 1985. Bone marrow aspirates showed a nucleated cell count of $147\times10^4/mm^3$, contained 92.8% blast cells, which were negative for POX, and were analyzed as lymphoblasts morphologically. He was diagnosed as ALL FAB L_1. Chromosome analysis showed unbalanced translocations that resulted in complete trisomy of the long arm of chromosome #1. The heterochromatic region of chromosome #1(1qh) was associated with the telomeres of whole chromosomes #1,#2, #3,#8,#9,#11,#14,#15,#16,#21,#22 in 100% of the 100 metaphases completely analyzed. Telomeric association in leukemic cells is a very rare event and this is only the third known report of its occurrence with ALL. We would like to give a name "Jumping translocation" to this new cytogenetic combination which may have a role in tumor etiology, as the whole arm of 1q seems to behave just like jumping to the telomeres of eleven different chromosomes.

A 29

CHROMOSOME ABNORMALITIES INVOLVING BAND 13q12 IN HEMATOLOGIC DISORDERS
Ichirou KAMINO, Yasunobu YOKOYAMA, Hiroko KOBAYASHI, Yoshimori ISHIHARA and Kazumasa HIKIJI(Div. Res. Lab., Special Reference Lab. Co. Inc., Hachioji)

It has been well-known that an interstitial deletion of band q14 from the long arm of chromosome #13 has been observed as a constitutional aberration in cases of the childhood neoplasm, retinoblastoma.
Then, this time, eighteen patients with hematologic disorders involving deletion of chromosome 13q have been studied with respect to the chromosomal breakpoint and the clinical-cytogenetic correlations, retrospectively.
Eighteen patient's(13 males, 5 females) ages ranged from 36 to 79 yr (mean age, 59.7) and there was an excess of males, respectively. Even though the most cases (15/18) could be diagnosed as preleukemic stages, the results of our studies were suggested that a chromosome #13 interstitial deletion may not be associated with any specific hematologic disorders or chemotherapy-related secondary leukemia.
Cytogenetically, on the basis of their breakpoints, no terminal deletions were observed and the cases were categorized into two types of interstitial deletions and a type of translocation: del(13)(q12q14), del(13)(q12q22) and t(13;13)(q12;q14). And 13q12 were involved common to all interstitial deletions and reciprocal translocation in our cases.

A 30

FURTHER ANALYSES OF c-MYB GENE IN T-CELL MALIGNANCIES WITH del(6q). Michiko OKADA, Masako SAITOH, Kura KUBOTA, Yoshiko NOMURA(Chromosome Lab., Shiseikai Dai-Ni Hosp., Tokyo), [1]Naotoshi KANDA, [2]Naohiko MASUDA, [2]Hideaki MIZOGUCHI([1]Dept. Anat., and [2]Div. Hematol., Tokyo Women's Med Coll., Tokyo)

Karyotype and c-myb gene analyses were made on two T-cell leukemia patients with del(6q) anomaly. The first case had a diagnosis of ATLL. Karyotypes of the PHA-stimulated blood cells were 46,XX/46,XX,dir dup(1)(pter→p36.3::p33→p31.1::p36.3→qter),del(6)(q21q23),-14,-16,+t(?;14)(?;q12→q22::q24→qter),+t(?:16)(?;q12.1→qter) (2/28). The other case had a diagnosis of T-ALL. Unstimulated blood and marrow cells showed the karyotypes of 46,XY/46,XY,del(6)(q21q27) (12/6 in the blood and 14/3 in the marrow). DNAs extracted from the fresh blood and marrow samples were digested in 7 ways using EcoRI,BglII,SacI,HindIII and BamHI,blotted and hybridized with c-myb-specific probes,Co 044 and 045 which were provided by JCRB. No alterations were found in these samples. Previously we obtained the same result on T-cell lymphoblastic lymphoma cell line with a del(6)(q21q27). These findings suggests that c-myb gene, assigned to 6q22-q24, is retained in malignant T-cells with del(6q) anomaly.

A 31

A CASE OF X/AUTOSOMAL TRANSLOCATION t(13;X) WITH UNILATERAL RETINOBLASTOMA Yoshitsugu YAMAMOTO, Hiroshi NAKAI, Kaoru TAKEDA, Keiya TADA, (Dept. Pediatr., Tohoku Univ., School of Med., Sendai)

The proband, a female, was born at term by normal delivery (birth weight 1,800g). She had accessory ears, anal atresia, vaginorectal fistura and bilateral inguinal hernias. At 2 years of age, she was reffered to us because of multiple anormalies, developmental delay and chromosomal abnormalities. High resolution banding showed her karyotype of 45,X,-X,-13,+der(13),t(13;X)(p11;q12). The translocated chromosome showed a late replicating pattern. Retinoblastoma was found in her right eye, and it was enucleated at 3-year-5-month-old. Metastasis or relapse of the retinoblastoma are not found till now.

A gene suppressing retinoblastoma (RB gene) is in the 13q14 region. In our case, two 13q14 bands must be kept intact in normal chromosome 13 and in the translocated chromosome. Since the latter showed the late replicating pattern, RB gene on this chromosome might be inactivated by the X chromosome inactivation system. Two probable mechanisms for tumorgenesis can be thought in this case; An additional new mutation to the RB gene on the intact chromosome, or loss of heterozygosity.

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GENETIC ANALYSIS OF FAMILIAL POLYPOSIS COLI (VI): TUMOR SUPPRESSION GENES FOR COLORECTAL TUMORS. Masayuki SASAKI[1], Kenji SUGIO[1], Mieko OKAMOTO[2], Chieko SATO[2], Michiko MIYAKI[2], Takehiko SASAZUKI[1]([1]Dept. Genet., Kyushu Univ., Fukuoka; [2]Dept. Biochem., Tokyo Med. Science, Tokyo)

Familial polyposis coli (FPC) is an autosomal dominant tumorigenic disorder, the major gene of which is mapped to chromosome 5q. We searched for a specific gene loss in colorectal tumors from FPC patients, as related to tumorigenesis by inactivation of tumor suppression genes. Loss of heterozygosity was observed on 17 chromosomes in colorectal tumors from FPC patients and on 7 chromosomes in non-polyposis colon cancer (NPCC). Frequent loss of heterozygosity in colorectal carcinomas from FPC patients was observed on chromosomes 5(21%), 12(21%), 17(39%) and 22(37%), and was also observed on chromosomes 5, 14, 17 and 22 in NPCC. Although loss of heterozygosity in adenoma from FPC patients was observed on 9 chromosomes, the frequencies were less than 10%. These result would suggest that tumor suppression genes for colorectal carcinoma may locate on chromosome 5, 12, 17 and 22, and that they may play a critical role in carcinogenesis, at least from adenoma to carcinoma. Moreover, the chromosomes in FPC patients may be unstable compared with those in NPCC patients.

A 33

SERUM TUMOR MARKERS IN DOWN SYNDROME

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In light of high frequency of complication of malignant tumors in Down syndrome(DS), tumor associated markers were examined in the sera from 36 patients with DS and were compared with those from age- and sex-matched controls. Of six markers examined, i.e., α1-acid glycoprotein(α1-AG), immunosuppressive acidic protein(IAP), carcino-embryonic antigen(CEA), α-fetoprotein(AFP), β2-microglobulin and ferritin, concentrations of α1-AG and IAP were significantly higher ($p<0.01$) in DS group than in control group. Concentration of α1-AG was inversely correlated with age($p<0.01$) in both DS and control groups, but that of ferritin and IAP in DS group correlated differently with age from control group. Discriminant function was constructed using patient's age and concentration of tumor markers except α-fetoprotein, and this function was able to discriminate the two groups at 84 % accuracy. These findings suggest that serum tumor markers are useful for screening tumor and for accurate prognosis in DS patients.

A 34

Down 症児におけるリンパ球表面抗原の解析. 後藤俊博・松田正利・大久保三郎 (シオノギバイオメディカルラボラトリーズ). ANALYSIS OF LYMPHOCYTES SURFACE ANTIGENS FROM THE CHILDREN WITH DOWN SYNDROME, Toshihiro GOTO, Masatoshi MATSUDA and Saburo OKUBO (Clin. Lab., Shionogi Co., Ltd, Osaka)

Down症児(DS)の白血病の発生頻度は非DSに比べ約20~30倍高いとされているが, その原因は不明である。そこでDSのリンパ球表面抗原を解析し何らかの特徴を有しているか検索した。生後2~60日までのDS 10名と非DS 20名の末梢血液を材料とし, CD2, 3, 4, 8, 20, 25 (抗IL-2 receptor), 38, 抗Transferrin receptor(Tf-R) および抗HLA-DRの各種モノクローナル抗体を用いてFlowcytometry により解析した。その結果, DSではT細胞マーカーであるCD2, さらに成熟T細胞マーカーであるCD3, およびCD4が非DSに比し有意 ($p<0.01$) に低値を示し, 相対的にCD8 が高値となった。これは細胞の分化成熟の問題とあわせてDSにおける免疫能の低下との関連を示唆するものと考えられた。また未熟T細胞マーカーにおいては, 非DSに比しTf-R が有意 ($p<0.05$) に高値となり, さらにCD 38も高値を示す傾向にあった。従って成熟マーカーの結果とあわせてDSのリンパ球はその分化段階において未熟な細胞がより多く存在しているものと考えられた。一方, 成熟B細胞マーカー (CD 20), DR抗原およびCD 25ではその差は認められなかった。今後は症例を蓄積しつつDSの免疫能の検索とリンパ球以外の細胞について表面抗原解析を実施し, より詳細な検索を実施してゆきたい。

A 35

EXPRESSIVITY OF A COMMON FRAGILE SITE, FRA(3)(p14). UNDER THE FOLATE DEFFICIENT CONDITION. Motoi MURATA, Mikako OTSUKA (Div. Epidemiol., Chiba Cancer Cent., Chiba), Ei-ichi TAKAHASHI and Tada-aki HORI (Div. Genet., Natl Inst. Radiol. Sci., Chiba)

Many common fragile sites are identified on human chromosomes, but their mechanism of expression is so far poorly understood. Among them, fra(3)(p14) is most commonly expressed under the culture condition of folate deprivation, and can be easily detected by conventional Giemsa staining. This study investigated inter-individual variation of its expressivity. Chromosome test was performed by 72 hrs of whole blood culture in folic acid- and thymidine-free Ham's M-F10 medium supplemented with 5% FCS and 2% PHA. Among 934 healthy blood donors (586 males and 348 females), expression frequency was varied from 0 to over 20%, forming a Poisson-like distribution. Remarkable sex (male>female) and age (younger>older) differences were observed. Seasonal variation with peaks in May and November was also observed. In 337 patients of benign and malignant diseases, these variations were unnoticed. It was also not correlated with family history of cancer, tobacco smoking, carrier state of any heritable fragile sites, radio-therapy within 3 months and affected diseases. On the other hand, it was highly correlated with the patient's unfavourable prognosis. It seems likely that these inter-individual variations are due to some non-genetic factors.

A 36

BRDU-DEPENDENT SPECIFIC GAP AT 11q23.1 IN EB VIRUS TRANSFORMED LYMPHOBLASTOID CELL LINES: Tatsuro IKEUCHI, Yoshiko TERUI (Dept. Cytogenet.) and Kohtaro YAMAMOTO (Dept. Virol. Immunol., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

When lymphoblastoid cell lines (LCLs) established by EB virus (EBV) transformation are treated with BrdU (10-100 μg/ml) for 24 hrs, achromatic gaps are specifically induced in a dose dependent manner at the distal region of chromosome 11q (11q23.1). Since this gap is not seen in PHA-stimulated lymphocytes, Sutherland et al.(1987) proposed the term"EBV modification site", which was registered as a gene symbol of EBVM1 at HGM9(1987). In order to know the generality of EBVM1 and to search for other possible modification sites, a total of 17 LCLs established in our laboratory from various sources were examined. In all the LCLs, EBVM1 was expressed in 20-90% of metaphases after BrdU (50, 100 μg/ml) exposure for 24 hrs. The expression rate was not related to the genetic sources of LCLs or to the in vitro age after transformation: even the LCLs cultured for 1-2 months after EBV infection showed high frequencies of EBVM1 expression. However, EBVM1 was not expressed in either B-lymphocytes cultured for 3 to 4 days after EBV infection, or spontaneously established EBNA-positive lymphoid cell lines. Further study is needed to understand the essential nature of EBVM1. So far, no other modification site than EBVM1 has been detected.

A 37

A NEW RARE FRAGILE SITE, FRA(11)(p15.1): COINCIDENCE WITH A BREAKPOINT OF t(7;11)(p15-p13;p15) IN ANLL PATIENTS. Ei-ichi TAKAHASHI[1], Yasuhiko KANEKO[2], Takaaki ISHIHARA[3], Masako MINAMIHISAMATSU[3], Motoi MURATA[4] and Tada-aki HORI[1] (1 Div. Genet., 3 Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba, 2 Dept. Lab. Med., Saitama Cancer Cent., Saitama, 4 Div. Epidemiol., Chiba Cancer Cent., Chiba)

Fragile site was analyzed on PHA-stimulated lymphocytes of acute non-lymphocytic leukemia (ANLL) patients with t(7;11)(p15-p13;p15) in leukemic cells. For the expression of fragile sites, cultures were treated with folate deprivation (M-F10), BrdU, distamycin A and Hoechst 33258. Fra(11)(p15.1) was induced by distamycin A and Hoechst 33258, but not in M-F10-, BrdU- and control-cultures. This fra(11)(p15.1) was coincident with one of the breakpoints of t(7;11)(p15-p13;p15). It may be suggested that this fra(11)(p15.1) play an important role in the induction of the chromosomal rearrangements of the t(7;11). In our population survey, distamycin A-inducible fra(11)(p15.1) was found neither in healthy Japanese subjects(0/845), nor in the patients with leukemia and hematologic disorders without the t(7;11)(0/239). From these results, this fra(11)(p15.1) can be newly classified as a rare distamycin A-inducible fragile site.

A 38

FREQUENCY OF RARE FRAGILE SITES AMONG CANCER PATIENTS. Hisako OCHI and Shaw WATANABE (Epid. Div., Natl. Cancer Center Res. Inst., Tokyo)

The chromosomal fragility of the lymphocytes from 300 patients, who were admitted to National Cancer Center Hospital, was studied. Pathological diagnosis of these patients were as follows; 95 breast cancers (BrC), 80 lung ca. (LC), 47 uterus ca. (UC), 11 ovarian ca. (OC), 28 bladder ca. (BlC), 15 other cancers and 24 benign tumors(BT). Three induction methods for rare fragile sites were employed, i.e. (1) folic acid and thymidine depletion, (2) addition of distamycin A and (3) addition of bromodeoxyuridine (BrdU). Nineteen carriers of rare fragile sites (FS) were found: folate sensitive fra(11)(q13) was detected in 1 patient(pt) with LC, folate sensitive fra(16)(p12) in 1 pt with LC, distamycin A inducible fra(16)(q22) in 3 pts with LC, 1 each with BrC, BlC and BT, distamycin A inducible fra(17)(p12) were detected in 4 pts with LC, 1 each with BrC, OC, UC, BlC and psuedolymphoma, and BrdU requiring fra (10)(q25) was seen in 1 each with BrC and BT. Thus, the frequency of rare FS was 6.5% in 276 pts with cancers and 8.3% in 24 pts with BT, revealing almost same as healthy Japanese population (6.0% by Takahashi et al.). However, the frequency was 11.3%(statistically higher than the healthy; $p<0.05$) in 80 pts with LC, suggesting the pts with rare FS may have predisposition to develop some kind of cancer.

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RARE FRAGILE SITES IN A MENTALLY RETARDED JAPANESE POPULATION. III.
Kunikazu KISHI (Sch. Hlth Sci., Kyorin Univ., Tokyo), Akira HOMMA and Riichi IMAMURA (Sec. Psychiat., Tokyo Metropolitan Inst. Gerontol., Tokyo)

We have carried on a survey of carriers with fragile X (fra X) chromosome and autosomal fragile sites (AFS) in Japanese institutionalized mentally retarded patients. The purposes of the study are to investigate the frequency of fra X syndrome and to compare the frequency of carriers with AFS between karyotypically normal and abnormal patients. To detect all the three groups of rare fragile sites (FS): i.e. folate sensitive, distamycin A inducible and BrdU requiring FS, blood samples from patients were treated in the following three ways: the culture in TdR and folic acid-free Ham's F10 medium for 72 h, the treatment with 50 ug/ml distamycin A for 24 h and the treatment with 7 ug/ml BrdU for 24 h. The frequency of fra X syndrome was 5.7 % in karyotypically normal male patients (12/209). Carriers with AFS in 300 karyotypically normal and 64 karyotypically abnormal patients were 17 (5.7 %) and 8 (12.5 %), respectively. The present result support the view that AFS may induce chromosome rearrangements or nondisjunction. Further investigations, both population and familial studies, will be required for clarifying the clinical implication of AFS.

A 40

FIBROBLAST-SPECIFIC COMMON FRAGILE SITES INDUCED BY APHIDICOLIN.
Ichiro MURANO, Akira KUWANO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med. Ube)

The distribution and frequency of aphidicolin-induced common fragile sites were studied in chromosomes of cultured skin fibroblasts and PHA stimulated PB lymphocytes from five normal individuals ranging in age from 24 to 35 years, with 0.2 μM aphidicolin added for the last 26 h of culture. Fragile sites frequent in fibroblasts from normal individuals were 3q26.2 (23.6% of the total breaks), 7q11.23 (12.8%), 16q23 (10.3%), 1p31 (6%), 10q11.2 (4.6%), 12q23 (4.6%) and 7q31 (3.5%), while those in lymphocytes from the same individuals were 3p14 (18.1%), 16q23 (13%), Xp22 (6.9%), 7q32 (5.3%) and 14q24 (4.2%). The number of breaks and gaps in 100 metaphases was 36.8 in fibroblasts and 279 in lymphocytes from the same individuals. Their rates of chromosome-type breaks and gaps were 7.9% and 54.5%. Thus, the distribution and frequency of aphidicolin-induced fragile sites were different between skin fibroblasts and PB lymphocytes, possibly reflecting their difference in DNA replication sequence or gene activity.

A 41

Common fragile sites in EB-stimulated B lymphocytes. Akira KUWANO, Yoshitsugu SUGIO, Ichiro MURANO, and Tadashi KAJII (Dept. Pediat., Yamaguci Univ. Sch. Med. Ube)

The frequency and distribution of fragile sites were studied in EB virus-stimulated B lymphoblastoid cells from six normal individuals, one male and five females aged 24 to 33 years. Aphidicolin, 0.2 μM, was added to culture 26 hours before harvest. Spontaneous breaks in control cultures ranged from 4 to 12 in 100 metaphases. The avarage rate of gaps and breaks in aphidicolin treated cultures was 90 in 100 metaphases. The most frequent fragile site was 3p14(34.9% of total breaks), followed by 16q23(12.8%), 7q11.2(6%), 4q23(5.4%), Xp22.2(2.9%), 9q13(2.4%) and 11q23.1(2.4%). The sites at 3p14, 16q23 and Xp22.2 have been described in PHA-stimulated T lymphocytes, that at 7q11.2 has been identified in skin fibroblasts and bone marrow cells, and that at 11q23.1, EBVM1, occurs spontaneously in EB-virus stimulated B lymphocytes (Sutherland et al. 1987). The site at 9q13 has not been reported. It was observed spontaneously in 0-2% of lymphocytes, and 1-6% of aphidicolin-treated lymphocytes.

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INVESTIGATION OF MICE TESTIS SPECIFIC ANTIGEN. Osamu MIKAMI, Hiromi SAKAMOTO, Yoshihiro YAMAMOTO, Masafumi HANDA and Jun-ichi FURUYAMA (Dept. Genet.,Hyogo Coll. Med., Nishinomiya)

Female mice of the C57BL/6 were immunized with a homogenate from mice testis; cell fusion of spleen cells and P3U1 cells yielded two hybridomas producing IgM. Specificity of monoclonal antibody (Moab) in ascitic fluid was investigated by indirect immunofluorescence, using frozen sections. Testicular antigen (TA) was prepared by tissue sonication, and then subjected to column chromatography. TA detected in the void volume by ELISA was analyzed by SDS-PAGE and Western blotting. Immuno-staining of membrane filters revealed a broad area within the 45K - 205K daltons range. TA in the void volume was treated with proteolytic enzymes to determine its nature, but no changes were observed after Western blotting. From these results, TA can be thought of as being a rather complex molecule more than a simple protein. Thus, TA in the void volume fraction was digested by peptidases and glycolytic enzymes besides protease; electrophoreseed using cellulose acetate membranes and immuno-stained with Moab 1A1. Evidences obtained from these experiments strongly suggest that TA consists of an acidic peptide and carbohydrate molecule. The antigenicity would be included in the carbohydrate epitope. Moreover, partial digestion of TA by β-galactosidase indicates that the lacto-series structure is included in the antigenic carbohydrate moiety.

A 43

Y SPECIFIC REPETITIVE SEQUENCE FOUND IN THE LARGE SATELLITE OF CHROMOSOME 13. Hiromi SAKAMOTO, Masahisa HAGIWARA, Yoshihiro YAMAMOTO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med., Nishinomiya) Yoshie SUGAHARA, Hiroko MIMURA (Dept. Clin. Genet., Hyogo Coll. Med., Nishinomiya) and Yutaka NAKAHORI (Dept. Congen. Abnorm. Res., Natl. Child. Med. Res. Cent., Tokyo)

Mother and the son whose chromosomes 13 had large satellites were found. These satellites showed brilliant fluorescence stained with quinacrine mustard. We tried in situ hybridization using two probes derived from pHY2.1 and pHY10, which had been cloned from Y chromosome specific repetitive sequences. After ordinary preparation of slides for chromosomal analysis, chromosomal DNA was denatured dipping in 70°C 70% formamide for 2 minutes. Probes were labeled with biotinylated dUTP by nick translation. After hybridization at 42°C for overnight, the slides were washed under a high stringency condition. They were treated with avidin and biotinylated alkaline phosphatase. Biotin-labeled DNA probes which had hybridized with chromosomal DNA were visualized by developing formazan. Formazan was recognized on the large satellite of chromosome 13 and distal portion of long arm of Y chromosome. We revealed that DNA sequences in these regions were very similar.

A 44

Cloning of Y-specific unique DNA sequences from flow sorted Y-chromosome specific λ-phage library. Yutaka NAKAHORI, Takashi TAMURA, Masao YAMADA and Yasuo NAKAGOME (Dept. Congen. Abnorm. Res., Natl. Child. Med. Res. Cent., Tokyo)

A plasmid (pYN87006), which has two copies of the 3.4Kb Y-chromosome specific repeated sequence in the reversed direction, has unique characteristics. It is infertile in DH1 without DNA fragment inserted between the repeated sequences. It is also infertile when another DYZ1 sequence is inserted. Using these characteristics, we have subcloned DNA fragments from ATCC Y-chromosome specific EcoRI library and obtained 29 independent clones. Among them, we found unique sequences in 27 clones (31 fragments) and performed Southern hybridization analysis against DNAs of normal males and females. Eleven clones revealed to be derived from Y-chromosome.

A 45

Analysis of structurally abnormal Y-chromosomes using Y-chromosome specific DNA fragments. Takashi TAMURA, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (Dept. Congen. Abnormal. Res., Natl. Child. Med. Res. Cent., Tokyo)

DNAs derived from individuals who have an structurally abnormal Y-chromosome have been analysed by Southern hybridization with the Y-specific probes. The probes used in this study were as follows; 47Z, 52d, 50f2 and 49f kindly provided by J.Weissenbach and Y10, 4a, 6c, 7a, 17a, 19a, 24a, 26a,b, 27a, 28a and 31a cloned in our laboratory. The order of these probes were determined in detail; from distal short arm to long arm (47z, 52dB, 52dC), (50f2A,B, 50f2D, 7a, 17aL, 27a, 4a), 28a, 26a,b, 24a, (31a, 19a),(49f,52dA,50f2C,E), 17aU, 6c, Y10. The relationship between the sexual differentiation and the presence or absence of a particular segment of Y has not been established yet.

A 46

Behavior of DNA fragments on human Y-chromosome in the primate evolution. Satoko YAMAGATA, Yutaka NAKAHORI, Takashi TAMURA, Masao YAMADA, Yasuo NAKAGOME (Dept. Congen. Abnormal. Res., Natl. Child. Med. Res. Cent., Tokyo) and Osamu TAKENAKA (Primate Res. Inst., Kyoto Univ., Inuyama)

Eleven human Y-chromosome specific probes cloned in our laboratory were hybridized to the male and female primate DNAs and rodent DNAs to study when and where, in terms of evolution, the DNA fragment appeared and disappeared from the Y-chromosome. In fact, many of them varied in their origin and divergence. 87-4a was best preserved. It showed X-Y homology in human genome and has been conserved even in mouse, rat (X-chromosome specific) and ox (X-Y homologous). On the other hand, Y10 revealed extensive polymorphism of autosomes in many kinds of primates, although it was Y-specific in man. It was found very useful in the individual identification in the Japanese monkeys.

A 47

INDUCTION OF CHROMOSOME REARRANGEMENTS IN G_1 LYMPHOCYTES FROM XP PATIENTS BY ARABINOFURANOSYLCYTOSINE (ARA C).
Kunikazu KISHI (Sch. Hlth Sci., Kyorin Univ., Tokyo)

It has been considered that the induction of chromosome rearrangements by inhibitors of DNA polymerase α results from inhibition of repair DNA replication. I have shown that whereas arabinofuranosylcytosine (ara C) induces chromosome rearrangements in G_1 lymphocytes which were not treated with any mutagen, aphidicolin (APC) does not. This result suggests that mechanism other than repair may be involved in the induction of chromosome rearrangements by ara C. In the present study, involvement of UV endonuclease was investigated using lymphocytes from XP patients of complementation group A. Peripheral lymphocytes which were either treated or untreated with 4NQO in their G_0 phase were cultured in their G_1 phase in the presence of ara C or APC, and induced dicentrics and rings (dic & ring) in the first metaphases after culture initiation were scored. 4NQO pretreatment raised the frequencies of dic & ring in normal lymphocytes, but not in XP ones. However, ara C did induce dic & ring in XP cells which were not pretreated with 4NQO. The present result shows that UV endonuclease will be involved in repair mediated-chromosome rearrangements, but not in ara C-induced rearrangements.

A 48

SISTER CHROMATID EXCHANGE FREQUENCIES IN LYMPHOCYTES FORM PATIENTS WITH ALCOHOLICS.
Kunihiko Miura and Kanehisa Morimoto (Dept. Hygiene and Preventive Medicine, School of Medicine, Osaka University)

Excessive alcohol consumption has already been known to increase the incidence of a variety of diseases including cancer of the alimentary tract and the colon. Ethanol has been shown to be clastogenic or to lead to abnormal meiotic chromosomal behavior in plant cells. In human cells, however, reports on the clastogenic effects of ethanol are inconsistent.

No increase of sister chromatid exchange (SCE) frequencies was reported in human lymphocytes when treated with ethanol in vitro, while treatment with acetaldehyde, the first metabolite of ethanol, induces SCEs in human lymphocytes as well as in human fibroblasts.

In the present study, we investigated the baseline, or MMC- or 4NQO-induced SCE frequencies in peripheral blood lymphocytes from abstinent alcoholism patients. The results show that clear decrease in baseline SCE frequencies in time-related manner up to 60 days in abstinence.

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MMC INDUCED SCEs AND CELL CYCLE KINETICS IN LYMPHOCYTES FROM PATIENTS WITH KLINEFELTER SYNDROME. Zentaro Yamagata, Sumio Iijima, Tatsuya Takeshita, Chiaki Ariizumi (Dept. Hlth. Sci, Yamanashi Med. Col., Yamanashi) and Makoto Higurashi (Dept. Maternal and Child Hlth, Univ. Tokyo, Tokyo)

The chromosomal sensitivity to MMC and cell cycle kinetics in cell from patients with klinefelter syndrome, sex chromosomal disorders and high risk of malignant tumor, were studied by techniques of SCEs. Heparinized peripheral blood samples were obtained from four patients with klinefelter syndrome and four normal karyotypical control. MMC were added each culture to give the appropriate final concentrations (0, 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7}M) for the entire culture period. The frequencies of MMC induced SCEs increased in proportion to the increase of MMC concentrations in both patient and normal control cells. At low levels of MMC there were no significant difference in SCEs frequencies between the patient and normal control cells, but at 3×10^{-8} M ($P<0.05$) and at 1×10^{-7}M ($P<0.01$) MMC concentrations a significant increase in frequencies of MMC induced SCEs was observed in cells from patients in comparison with the value in cells from normal controls. The analysis of cell cycle kinetics induced MMC revealed that in patients cells dividing for 1st and 2nd times were fewer and 3rd times were more than in normal control.

A 50

ACTIVITY OF DNA LIGASE I IN BLOOM'S SYNDROME LYMPHOBLASTOID CELLS. Takayuki KURIHARA[1], Kouichi TATSUMI[2], Hirobumi TERAOKA[3], Masao INOUE[1] and Hiraku TAKEBE[2] ([1]Cent. Res. Lab., Kanazawa Med. Univ., [2]Dept. Mol. Oncol., Fac. Med., Kyoto Univ., [3]Dept Pathol. Biochem., Med. Res. Inst., Tokyo Med. Den. Univ.)

In order to verify that Bloom's syndrome (BS) results from a defect in DNA ligase I which is indispensable for semiconservative DNA replication, we have compared the activity of this enzyme in crude extracts between lymphoblastoid cell lines (LCLs) derived from patients with BS and those from normal controls. Sonicated extracts were chromatographed on hydroxylapatite column, and each fraction was assayed for DNA ligase I by using poly(dA)·oligo(5'-^{32}P-dT) as a substrate. The enzyme activity in GM3403C, which originated from an Ashkenazi patient with BS, was approximately one half of that in normal control LCLs, confirming the original finding made by Willis and Lindahl. A representative Japanese BS LCL with high frequency of sister-chromatid exchange (SCE), BSL-2KA, contained the enzyme activity indistinguishable from that in control LCLs. In this regard BSL-2KA resembles to an Anglo-Saxon BS LCL, Wl032, with which an abnormal size of DNA ligase has recently been reported by Willis et al. These data collectively suggest that BS is probably the result of a heterogeneous group of mutations.

A 51

DIFFERENT MUTATIONS RESPONSIBLE FOR THE ELEVATED SISTER CHROMATID EXCHANGE FREQUENCIES IN BLOOM SYNDROME AND X-IRRADIATED B-LYMPHOBLASTOID CELL LINES ORIGINATED FROM ACUTE LEUKEMIA. Yukimasa SHIRAISHI, Takahiro TAGUCHI (Dept. Anat., Kochi Med. Sch., Kochi)

Cell hybridization and Co-cultivation protocols have been used to determine whether the increased rates of sister chromatid exchanges (SCEs) exhibited by Bloom syndrome (BS) and a human mutant cell line (CCRF-SB-T1), originated from X-irradiated acute leukemia derived B-lymphoblastoid cell line, have the same or different bases. Cell fusion of CCRF-SB-T1 with each of 4 different BS B-lymphoblastoid cell lines (LCLs), retaining high SCE character, exhibited low (normal level) numbers of SCEs, signifying complementation. Co-cultivation of CCRF-SB-T1 and BS B-LCLs also resulted in the significant reduction of SCE from 70 to 35 level in BS cells, and lowered the BrdU concentrations from 15 μg/ml (0.05 mM) to 2.0 μg/ml (0.01 mM) necessary for sister chromatid differential staining (SCD) and resulted in completely normal level of SCE in CCRF-SB-T1 cells. This strongly suggests that the defects in the two cell types are different.

A 52

ABNORMAL INDUCTION OF SISTER CHROMATID EXCHANGES AND HOMOLOGOUS CHROMATID EXCHANGES BY THYMIDINE DEPRIVATION IN BLOOM SYNDROME CELLS. Hideo TSUJI, Tada-aki HORI (Div. Genet., Natl. Inst. Radiol. Sci., Chiba), Mickael W. HEARTLEIN and Samuel A. LATT (Genet. Div., The Children's Hosp., Boston)

It is known that thymidine deprivation can induce recombination in bacteria and yeast. To investigate the effect of thymidine deprivation on the induction of chromosomal abnormalities in mammalian cells, Bloom syndrome(BS) or normal human lymphoblastoid cell lines were treated with low concentrations of thymidine or 5-bromodeoxyuridine for one cell cycle under the block of de novo thymidylate synthesis and then cultured further in the presence of a sufficient amount of thymidine or 5-bromodeoxyuridine. Sister chromatid exchanges (SCEs) and homologous chromatid exchanges (HCEs) were markedly induced by thymidine deprivation in BS cells mainly at later times after one cell cycle treatment, whereas normal cells exhibited a slight induction of SCEs and HCEs. Aphidicolin caused the reduction of SCEs and HCEs induced by thymidine deprivation. Addition of deoxyuridine under thymidine deprivation resulted in the enhanced induction of SCEs and HCEs in BS cells. These results suggest that thymidine deprivation is recombinogenic in mammalian cells, particularly in BS cells. It is also possible that deoxyuridine misincorporation under thymidine deprivation plays a role in the induction of SCEs and HCEs in BS cells.

A 53

DO REARRANGEMENTS INDUCE MEIOTIC NONDISJUNCTION OF UNRELATED CHROMOSOMES? Shin-ichi SONTA (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

The relationship between chromosome rearrangements and nondisjunction of unrelated chromosomes was studied by the use of Chinese hamster strains with reciprocal translocations and inversions. First, nondisjunction at meiosis I in male and female heterozygotes of these rearrangements was calculated from MII counts. The results indicated that there was no significant difference in the frequency of nondisjunction between normal (+/+) males and heterozygous (T/+) males, and between +/+ females and T/+ females, respectively. The frequency of nondisjunction at meiosis I in females was slightly higher than that in males. Second, nondisjunction at meiosis II in both sexes was calculated from chromosome counts of the first mitotic metaphases of embryos from the crosses between +/+ and T/+ animals. The results revealed that the frequencies of nondisjunction calculated form +/+♀ × T/+♂ embryos, and from T/+♀ × +/+♂ embryos were similar to that expected from +/+♀ × +/+♂ embryos. These findings suggest that the presence of chromosome rearrangements such as reciprocal translocations does not induce nondisjunction of chromosomes unrelated to the rearrangements.

A 54

ANEUPLOIDY IN HUMAN SPERMATOZOA (1ST REPORT): Yujiroh KAMIGUCHI, Hiroyuki TATENO and Kazuya MIKAMO (Dept. of Biol. Sci., Asahikawa Med. Col. Asahikawa)

Using our interspecific in vitro fertilization system between human spermatozoa and zona-free hamster ova, we studied spontaneous incidences of aneuploid spermatozoa and determined the chromosome groups to which the aberrant chromosomes belong. This attempt is to clarify the non-selected primary incidence of chromosome aberrations in zygotes due to nondisjunction of paternal origin.

(1) Total number of 7240 spermatozoa from 21 donors having normal semen quality were karyotyped. Number of aneuploid spermatozoa was 99 (1.4 %), including 49 (0.7 %) of hyperhaploidy and 50 (0.7 %) of hypohaploidy. This incidence was the lowest as compared with the data of 5 previous studies.

(2) Nondisjunctional chromosomes observed were classified into 8 chromosome groups(A-G and Y). Their incidences were 17 (15.0 %) in group A, 9(8.0 %) in B, 39 (34.5 %) in C, 8 (7.1 %) in D, 14 (12.4 %) in E, 9 (8.0 %) in F, 14 (12.4 %) in G, and 3 (2.7 %) in Y chromosome. These incidences were compared with the theoretical value which was calculated according to the assumption that nondisjunction had taken place at equal incidence in all chromosome groups. The observed incidences coincided well with the theoretical value.

A 55

EFFECTS OF γ-RAYS ON HUMAN SPERM CHROMOSOMES. Hiroyuki TATENO, Yujiro KAMIGUCHI and Kazuya MIKAMO (Dept. Biol. Sci., Asahikawa Med. Col., Asahikawa).

It has been possible to study effects of mutagens on human sperm chromosomes by use of interspecific in vitro fertilization system with zona-free hamster ova. We reported already effects of X-rays to show that human sperm chromosomes are highly radiosensitive (Kamiguchi et al., 1987).

In this study, we examined effects of γ-rays and compared them with those of X-rays. Semen samples from five healthy men were exposed to 0.27, 0.54 and 1.08Gy ^{137}Cs-γ-rays. Incidences of spermatozoa with induced chromosome aberrations were 13.1%, 26.8% and 40.1%, respectively. The incidences increased linearly with increase of the doses (Y=2.58+36.73D). Regardless of the doses, more than 70% of chromosome aberrations were breaks and fragments of chromosome-type. Breaks and fragments of chromatid-type and exchanges of both types occurred but less frequently. Incidences of these aberrations increased also linearly. There was no significant difference between γ-rays and X-rays with respect to capacity of inducing chromosome aberrations in human spermatozoa. This result indicated that relative biological effectiveness (RBE) of γ-rays was unexpectedly high in human sperm chromosomes, as compared with the RBE in somatic cells.

A 56

CYTOGENETIC CHARACTERISTICS OF CULTURED LYMPHOCYTES FROM PATIENTS WITH DOWN'S SYNDROME. Tatsuya TAKESHITA, Hitoshi HOSHINO, Chiaki ARIIZUMI, Sumio IIJIMA(Dept. Health Sci., Yamanashi Med. Col., Yamanashi), and Makoto HIGURASHI (Dept. Maternal Child Health, Univ. Tokyo, Tokyo)

Higher sensitivity of lymphocytes from patients with Down's syndrome is well-known. We investigated, in the present studies, sensitivities of Down's syndrome lymphocytes to several mutagens, indicated by frequencies of sister chromatid exchange (SCE). When exposed to 0, 10, 20, and 40 uM of 3-methylcholanthrene, SCE frequencies in the lymphocytes from patients (8.2, 10.9, 12.2, 12.7) were not different from those from controls (8.0, 11.5, 10.5, 14.4). The results were inconsistent with the previous report. As for the exposures to mitomycin C ($3X10^{-8}$, $6X10^{-8}$ M) or vinyl acetate (0.3, 0.6 mM), the lymphocytes from patients exhibited similar SCE inductions compared to those from controls. When the lymphocytes from patients and controls in their 40's were exposed to X-ray , frequencies of dicentric plus ring chromosomes in patients were 1.5 times higher than those in controls at the dose of 500 rads, although the difference was not significant (t-test; p=0.054).

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EFFECTS OF MAGNETIC FIELD ON THE X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN LYMPHOCYTES FROM THE PATIENTS WITH DOWN SYNDROME. Sumio IIJIMA, Tatsuya TAKESHITA, Zentaro YAMAGATA (Dept.Hlth.Sci., Yamanashi Med.Col., Yamanashi), and Makoto HIGURASHI (Dept.M.C.H.,Fac.Med.,Univ.Tokyo,Tokyo)

Frequencies of chromosomal aberrations(dicentrics and rings) were examined in the Down lymphocytes and the normal lymphocytes after exposure of X-ray at the doses of 100, 200, and 400 rads and/or magnetic field ($8x10^{3}$G). The frequencies of dicentrics and rings per cell increased with the increasing doses of irradiation both in the patients and the controls. However, there were no significant differences between the frequencies of dicentrics and rings of Down lymphocytes and those of control lymphocytes at all doses examined. When cells were treated with X-ray and magnetic field, the significant enhancement in number of dicentrics and rings was observed neither in the normal cells nor in the trisomic cells.

A 58

CHROMOSOME ABERRATIONS IN THE PRENATALLY EXPOSED A-BOMB SURVIVORS, HIROSHIMA. I. 105 CASES IN THE DISTALLY EXPOSED GROUP.
Kazuo OHTAKI and Akio AWA (Department of Genetics, RERF, Hiroshima)

In the course of cytogenetic surveys on prenatally exposed A-bomb survivors in Hiroshima and Nagasaki, we were able to score 11,164 G-banded metaphases derived from cultured lymphocytes of 105 survivors who were distally exposed while in utero with the estimated DS86 kerma of less than 0.005 Gy, thus served as a comparison group for the proximally exposed. Of the 11,164 metaphases analyzed, 326 cells were found to carry structural rearrangements of chromosomes. Among the aberrations detected, reciprocal translocation were the most common type of abnormalities, 149 in 379 aberrations. It is noteworthy that chromosomes No. 7 and No. 14 were involved highly frequently in the formation of translocations, which is known to be a common feature of spontaneous (or background) chromosome aberrations observed in the non-irradiated persons.

A 59

CYTOGENETIC STUDY IN 16,298 CHILDREN OF A-BOMB SURVIVORS, HIROSHIMA AND NAGASAKI.

Akio A. AWA, Mimako NAKANO (Dept. Genet., RERF, Hiroshima), Takeo HONDA and Masahiro ITOH (Dept. Radiobiol., RERF, Nagasaki)

The present report deals with the results of a cytogenetic survey, undertaken during the period between 1967 and 1985, on 16,298 children born to atomic bomb survivors and their controls in Hiroshima and Nagasaki (8,322 in the proximally exposed group, and 7,976 in either distally exposed or non-exposed group). Chromosome abnormalities detected in this survey were found to consist mainly of sex chromosome abnormalities and autosomal structural rearrangements. It is thought that there should be relatively little selection due to early infantile death against individuals with balanced autosomal abnormalities.

The results indicated that there was no statistically significant difference in overall frequencies of cytogenetically abnormal cases between the exposed (0.52%, or 43 in 8,322) and comparison (0.64%, or 51 in 7,976) groups. Two de novo mutants were identified through family studies on the probands with translocations and inversions of the balanced type. The mutation rates for these abnormalities were estimated to be 0.98×10^{-4} per gamete per generation in the exposed group, and the same value of 0.98×10^{-4} in the comparison group.

A 60

山陰地区における Down 症候群の合併症と予後　家島　厚[1]、竹下研三[2]、大谷恭一[3]、安東吾郎[3]（[1]鳥取県立皆生小児療育センター小児科、[2]鳥取大学医学部脳研神経小児科、[3]鳥取県立中央病院小児科）. COMPLICATIONS AND PROGNOSIS OF DOWN SYNDROME IN SAN-IN DISTRICT. Atsushi IESHIMA[1], Kenzo TAKESHITA[2], Kyoichi OHTANI[3] and Goro ANDO[3] ([1]Dept. of Pediatrics, Tottori Prifectural Kaike Rehabilitation Center for Disabled Children, Yonago; [2]Div. of Child Neurology, Tottori University School of Medicine, Yonago; [3]Dept. of Pediatr., Tottori Prifectural Central Hospital, Tottori).

Down症候群（以下ＤＳと略）の合併症についての地域ベースの報告はまれである。昭和４２年から６２年に出生した鳥取県のＤＳの年次的頻度を求め、合併症の頻度と予後との関係を検討した。死亡例もふくめたＤＳの頻度は５年毎に集計すると昭和４８年以後は出生1000に0.9と安定していた。昭和４８年以後の合併症の頻度を求めると、心奇形は35.9 %、鎖肛4.3 %、十二指腸閉鎖2.6 %、白血病3.4 %、けいれん2.6 %だった。生存例における心奇形の頻度は、加齢に伴い減少傾向がみられ、５才毎に順に41.7 %、25 %、10.5 %と減少した。死亡時年齢と死因の関係を検討すると、１才までは心奇形による死亡が多く、その後は肺炎、白血病での死亡が多かった。心奇形の合併により、有意に独歩の遅れる傾向が見られたが、有意語がでる時期には有意差がなかった。

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A CYTOGENETIC SURVEY OF CONSECUTIVE NEWBORN INFANTS.
Akira MATSUNOBU, Michiko OHNO, Tomoko FUNATO, Kyoko YOSHIHARA, Noriko,YABE, Tohru MAEDA (Kitasato Univ. Hosp., Kanagawa), Hisashi HAGIWARA (Kyorin Univ., Tokyo)

Cytogenetic investigations have been carried out with particular concern to the frequency of chromosome abnormalities in newborn infants. This is a report of data derived from 15,394 consecutive liveborns (7,906 males and 7,488 females) during a period from 1975 to 1986. Of these, 99 infants (0.64%) showed a major chromosome abnormality. Thirty infants (0.19%) had a sex chromosome abnormality. Male sex chromosome abnormalities occurred with a frequency of 0.20%, and 0.19% in females. Most of the babies with sex chromosome abnormalities were physically normal at birth. Twenty-nine infants (0.18%) were autosomal trisomics: 21 with a trisomy 21, 6 with a trisomy 18, and 2 with a trisomy 13. Five infants had unbalanced structural rearrangements. All these showed characteristic clinical features for each karyotype. Marker chromosome (supernumerary small metacentric chromosomes) were detected in four infants. Thirty-one infants (0.20%) had balanced autosomal rearrangements: 15 with a robertsonian translocation, 14 with a reciprocal translocation, and 2 with a pericentric inversion.

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UNEXPECTED CHROMOSOME ANOMALIES DETECTED IN PRENATAL DIAGNOSIS.
Kodo SATO, Yoshiko MORITA, Noriko TAKANO (Dept. Ob. Gyn.,Toranomon Hosp., Tokyo), Masumi OGAWA (Dept. Hemat.,Toranomon Hosp.,Tokyo)

Unexpected chromosome anomalies detected in prenatal diagnosis,such as 45,XO in a case with a previous child of regular 21 trisomy, raise some difficulties in the treatment after the procedure.Genetic amniocentesis was carried out in 500 cases at Toranomon Hospital between April 1986 and August 1988. A total of 22 unexpected chromosome anomalies were detected by G-banding method. Those anomlies included 1 case of 47,XXX, 1 case of 47,XXY, 1 case of 45,XO, 2 cases of extra marker chromosomes, 1 case of balanced translocation, 4 cases of mosaic , 7 cases of inversion (9), and others. We did not notify the results to the parents when we found mosaics which seemed not to reflect the fetal karyotype. Also we did not notify about inversion (9). We notified the results in the case revealed to have a fetus with sex chromosome anomalies,extra marker chromosome, or balanced translocation. Some parents eagerly wanted to have legal abortion even when we explained the absolutely low possibility of developmental abnormalities. The datas concerning the developmental prognosis of fetuses with those chromosome anomalies were still limited and further data collection was suggested to be needed for appropriate genetic counselling.

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CYTOGENETIC AMNIOCENTESIS FOR 1,011 CASES. Kaoru HANATANI, Nobuhiko HOSHI, Norio WAKE, and Seiichiro FUJIMOTO (Dept. Obstet. Gynecol., Hokkaido Univ., Sapporo)

Cytogenetic amniocentesis for prenatal diagnosis was performed on 1,011 cases since Jan.,1976 until Dec.,1987. Thirty-nine cases showed various types of chromosomal anomaly, including 10 cases with balanced translocation. Accordingly, 29 cases (2.9%) were diagnosed as having chromosomally abnormal fetuses. These 29 cases were 10 cases of 21-trisomy, 13 cases of other autosomal anomaly and 6 cases of sex chromosome anomaly. The indications for amniocentesis and the incidences of chromosomal anomaly were as follows; 1) advanced maternal age: 571 cases (56.4%) - 17 cases (3.1%), 2) previous child with chromosome anomaly: 216 cases (21.3%) - 3 cases (1.4%), 3) structural chromosome aberrations in the parent: 27 cases (2.7%) - 3 cases (11.1%), 4) suspect of abnormal fetus because of polyhydroamnios, severe IUGR etc.: 66 cases (6.5%) - 6 cases (9.1%), and 5) others (previous malformed child, anxiety, chromosome aberration in the family, etc.): 131 cases (13.0%) - 0 case (0%). As for the yearly change of the indication, advanced maternal age significantly tended to increase in number, gaining a majority after 1983. No adverse complication of amniocentesis was found on both mother and fetus. Thus, in high risk pregnancy group for chromosomal anomaly, this cytogenetic amniocentesis seemed to become more and more important.

A 64

羊水検査をめぐる諸問題. 木田盈四郎（帝京大・医・小児科）SOME PROBLEMS OF THE AMNIOTIC FLUID ANALYSIS.Mitsushiro KIDA(Dept.Pediat.,Teikyo Univ.,Tokyo)

アンケート用紙を１１０施設に送付して、わが国で出生前胎児診断がどの位行われているか調査した。回答のあった６８施設のうち、羊水検査を行っていたのは、２９施設(42.6%)であった。検査は昭和４６年から開始されており、検査総数４８９６例のうち、染色体の異常は２０２例(4.1%)で、そのうち数異常は９５(47.0%)、構造異常は２４(11.9%)、転座は６０(29.7%)であった。実際に検査を担当している人の意識を調査すると、男５３名(72.6%)、女２０名(27.4%)のうち、研究者１０名(13.7%)、検査技師１１名(15.1%)、医師４９名(67.1%)であった。染色体培養に従事している４３名の経験年数平均は9.0年である。個人の精神的背景として、信仰（宗教）の影響を受けていないと答えたのは４８名(68.6%)で、仏教４名、(5.7%)、キリスト教９名(12.9%)、無信教８名(11.4%)、神道１名であった。胎児の命は受精卵から守ると答えたのは３０名(41.1%)、胎芽期からは１０名(13.7%)、生まれて生存可能の時期からは３０名(41.1%)であった。胎児の命を守るのは、親（母親、父親）としたのは３８名(52.1%)、自然の法則が３１名(42.5%)、社会（法律）が１３名(17.8%)、医師が９名(12.9%)である。現在、羊水検査は社会的に認知されていないとするものは６名(8.2%)。ほぼ認められているとするもの６６名(90.4%)であった。バイオテクニックの未来は輝いているとするもの６名(8.2%)、５１名(68.5%)は一抹の危惧を持っていた。この調査を行って、わが国でも、羊水検査に対する社会的に認知が必要であると考えた。

A 65

ULTRASOUND STUDY OF THE KIDNEY AND URINARY TRACT OF CHILDREN AGED 0-6 YEARS. Akira MATSUI, Nobuko TAKEZAWA (Dept. Pediat.,Isesaki Municipal Hosp.,Isesaki), Yuchi NARUSE* and Takeshi MATSUDA**(Dept. Community Med.*, and Anatomy**, Toyama Med.& Pharmaceut.Univ.,Toyama)

Ultrasound study of the kidney and urinary tract was performed on 67 healthy children aged 0-6 years. The anteroposterior and longitudinal diameters of the kidneys were found to be significantly correlated with postnatal age, body length, body weight, head circumference and abdominal circumference. The anteroposterior mean diameters were 21.5 mm at 0-1m, 30.6 mm at 1ylm-2y, 31.3 mm at 2ylm-3y, 32.3 mm at 3ylm-4y, 31.8 mm at 4ylm-5y, and 33.1 mm at 5ylm-6y. The respective longitudinal mean diameters were 46.9 mm, 64.8 mm, 68.5 mm, 72.1 mm, 76.3 mm, and 77.2 mm.

In other 8 children of the same age group, congenital or genetic disorders of the kidney and urinary tract were found by ultrasound.

In conclusion ultrasound is useful for assessment of postnatal growth of the kidney as well as diagnosis of congenital or genetic disorders of the kidney and urinary tract of children.

A 66

PRENATAL DIAGNOSIS OF PEROXISOMAL DISORDERS.
Nobuyuki Shimozawa, Yasuyuki Suzuki, Shigehiro Yajima and Tadao Orii
(Dept. Pediatr., Gifu Univ., Gifu)

Peroxisomal disorders including adrenoleukodystrophy and Zellweger syndrome are new group of genetic diseases which have single or multiple peroxisomal dysfunction, and for which there is no effective therapy. Therefore, we analyzed some peroxisomal functions biochemically using amniocytes and skin fibroblasts of patients with these disorders in order to diagnose prenatally. METHODS: The very long chain fatty acids (J Inher Metab Dis 1985;8:5), the activity for the oxidation of lignoceric acid (Proc Natl Acad Sci USA 1984;81:4203), the amounts of enzyme proteins of peroxisomal β-oxidation (Clin Chim Acta 1986;156:191), the activity of dihydroxyacetone phosphate acyltransferase (Biochem Biophys Res Commun 1984;120:179) and intracellular distribution of catalase (Biochem Biophys Res Commun 1984;123:1054) were measured as described before. RESULTS: All of these biochemical abnormalities were seen in Zellweger syndrome, but patients with adrenoleukodystrophy were normal without the very long fatty acids and lignoceric acid oxidation. DISCUSSION: We consider these biochemical findings are also of potential value for prenatal diagnosis of other peroxisomal disease such as infantile Refsum disease, neonatal adrenoleukodystrophy, rhizomelic chondrodysplasia punctata and single deficiency of peroxisomal β-oxidation enzyme.

A 67

PRENATAL DIAGNOSIS OF LESCH-NYHAN SYNDROME (LNS) WITH RFLP ANALYSIS

Tatsuro KONDOH* (Dept. Pediat., Nagasaki Univ., Nagasaki), Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ.), Kimiko BABA (Div. Pediat., Natl. Nagasaki Hosp.), Kaoru SUZUMORI (Dept. Gynec. Obstet., Nagoya City Univ., Nagoya), Michio KOBAYASHI, Yoshiro WADA (Dept. Pediat., Nagoya City Univ.)

We report the result of prenatal diagnosis on a fetus in a family at risk of LNS and carrier detection on the members of the other LNS family, with the RFLP analyses. Genomic DNA extracted from leukocytes of probands and their family members and that from chorionic villi at 9 weeks of gestation was digested by BamHI, TaqI and BglII. The digest was hybridized to 3 probes, pDSK1(HPRT-cDNA), DXS10 and DX13, respectively. In both of the probands, the HPRT gene was present, while an RFLP (25kb/20kb) was detected in the members of a family with a pDSK1/BamHI combination. The RFLP of a male fetus in this family was identical to that of the proband, indicating that the fetus was a patient. No RFLPs within the HPRT gene were found in any members of the other family, but a 7.0kb/5.0kb and a 5.4kb/2.0kb RFLPs were detected with the combinations of DXS10/TaqI and DX13/BglII, respectively. Segregation analyses of this family revealed that the elder sister of the proband was a heterozygous carrier of LNS.

A 68

MICRO DNA-EXTRACTION: BLOTTED BLOOD METHOD AND GEL BLOCK METHOD

Tetsuya HIROTA*, Tatsuro KONDOH (Dept. Pediat., Nagasaki Univ., Nagasaki), Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki)

We established a method for extracting genomic DNA from a small amount of whole blood, cultured amniocytes and chorionic villi to diagnose a newborn and/or a fetus at risk of a genetic disease. A series of blood amounts was mixed with low-melting agarose gel to make a 100 ul block. Blood in the block was digested by protease K and by endonucleases, and Southern hybridization was then performed. Suitable results were obtained at least in 0.25 ml blood. Another trial was the blotted blood method. A series of blood amounts was blotted with Whatman 3MM paper, No. 2 paper (Toyo Roshi) and the paper used for screening metabolic diseases in the newborn, and kept for 1-20 days at room temperature. Dried paper with blood was immersed in 0.85% NaCl and gently shaked for 4 hrs to detouch leukocytes. Further DNA extraction was according to the standard method. The amount of DNA extracted with the blotted blood method was roughly 1/4 to 1/3 of that directly from whole blood. A minimum amount of blood for suitable result of one Southern hybridization was 0.3 ml. Both the methods described here are applicable to the diagnosis of a disease of a patient from whom taking a large amount of blood is difficult, and to the transport of the blood material.

A 69

PRENATAL AND CARRIER DIAGNOSIS BY DNA ANALYSIS IN 21-HYDROXYLASE DEFICIENCY FAMILIES. Jun NAKURA[1], Tetsuro MIKI[1], Koujin KAMINO[1], Yuji TAKEMOTO[1], Toshio OGIHARA[1], Yuichi KUMAHARA[1], Shin-ichiro TAKAI[2], Ken HAYASHI[3], Takuma KONDO[4] ([1]Dept. Med. Geriat., [2]Second Dept. Surg., Osaka Univ., Osaka. [3]Dept. Obs. Gynec., Kyoto Univ., Kyoto. [4]3rd. Dept. Pediat., Child. Med. Cent. of Osaka City, Osaka)

21-Hydroxylase (21-OHase) deficiency is the most common form of congenital adrenal hyperplasia, with a frequency of about one per 15,000 to 20,000 births in Japanese. In the 21-OHase deficiency family, it is useful to detect the heterozygous carrier and to diagnose the fetus because an early prenatal diagnosis of 21-OHase deficiency would allow treatment to protect the female from masculinization and/or anticipation of a life threatening adrenal crisis at birth. We report here the prenatal diagnosis of 21-OHase deficiency and the heterozygous carrier detection by recombinant DNA techniques, mainly the linkage analysis with RFLPs detected by 21-OHase and C4 cDNA genes. Thirteen out of 19 siblings in 22 families were able to be diagnosed by DNA analysis. In one case, we found the recombination between the HLA genes and the 21-OHase B gene. Moreover, in the family having a patient with 21-OHase deficiency of salt-wasting type, we carried out the prenatal diagnosis by analysing DNA from amniotic cells at 18 weeks of gestation.

B 1

EXPRESSION AND REPLICATION OF HEPATITIS B VIRUS GENOME IN TRANSGENIC MICE. Ken-ichi YAMAMURA, Kimi ARAKI, Junichi MIYAZAKI (Inst. Med. Genet., Kumamoto Univ. Med. School.), Naohiro TOMITA, Osamu CHISAKA, Kenichi MATSUBARA (Inst. Mol. Cell Biol., Osaka Univ.)

We produced transgenic mice by microinjecting partial tandem duplication of the complete hepatitis B virus (HBV) genome into fertilized eggs of C57BL/6 mice. One of eight transgenic mice was a high producer for both HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) in the serum. The HBV genomes were transmitted to the next generation and these F1 mice also produced both HBsAg and HBeAg. The mRNAs 3.5 kb, 2.1 kb and 0.8 kb were detected in the livers and the kidneys of these mice. In addition, 0.8-kb RNA was detected in the testis. Single-stranded and partially double-stranded HBV DNAs were shown to be produced in the cytoplasm of the liver and kidney. These HBV DNAs were associated with the core particles, indistinguishable from nucleocapsid produced in an infected human liver. Viral genome DNA were detected in the serum. These results demonstrate that the HBV genome integrated into the mouse chromosome acted as a template for viral gene expression allowing viral replication.

B 2

EXPRESSION OF HUMAN SERUM AMYLOID P COMPONENT GENE IN TRANSGENIC MICE
Tomohisa Iwanaga[1], Shoji Wakasugi[1], Takeaki Inomoto[1], Masahiro Uehira[1], Shuji Onishi[2], Seiji Nishiguchi[2], Kimi Araki[1], Masashi Uno[1], Jun-ichi Miyazaki[1], Shuichiro Maeda[2], Kazunori Shimada[2] and Ken-ichi Yamamura[1*] (Institute for Medical Genetics[1] and Department of Biochemistry[2], Kumamoto University Medical School, Kumamoto)

We have produced seven transgenic mice which carry human amyloid P component (SAP) gene. The 3.0 kb human SAP genes containing about 840 bp of 5' and 1.5 kb of 3' flanking region were injected into fertilized eggs of C57BL/6 mice. In five of the seven transgenic mice, human SAP was detected in the sera. The human SAP mRNA was detected only in the liver. Amounts of human mRNA in the liver and serum concentrations of human SAP were roughly proportional to the copy number of the integrated gene. Human SAP production lowered the serum levels of mouse endogenous SAP. SAP is one of the major acute phase proteins in mouse but a stable plasma protein in man. With the intraperitoneal administration of lipopolysaccharide, both the mRNA levels in the liver and serum levels of human SAP were slightly elevated.

B 3

EXPRESSION OF THE HUMAN $^{A}\gamma/\beta$-GLOBIN COMBINED GENE IN TRANSGENIC MICE:
Hideaki TOJO, Masami KUBO(Inst. Lab. Anim. Sci., Toyama Med. Pharm. Univ., Toyama), Yasuyuki FUKUMAKI(Lab. Genetic Infrom., Kyushu Univ., Fukuoka), Nobuyuki KUROSAWA and Zen-ichi OGITA(Inst. Orient. Med.,Toyama Med. Pharm. Univ., Toyama)

To study the switching mechanism of the gene expression between the human $^{A}\gamma$- and β-globin genes, we introduced a human $^{A}\gamma$/β-globin combined gene construct containing a 3.3kb sequence(Hind III/Hind III) of the $^{A}\gamma$-globin gene and a 5.5kb sequence (Bgl II/Bgl II) of the β-globin gene into the germ line of mice. Five transgenic mice carrying multiple copies(2-50 copies) of the gene construct as head-to-tail tandem array were obtained. Two of the mice expressed tissue-specifically the foreign globin genes. The expression pattern of the integrated genes during the mouse development was examined by dot blot and Northern blot technique. The human β-globin gene was expressed like an adult mouse globin gene. The human $^{A}\gamma$-globin gene was expressed at the fetal stage and its expression was sustained at the adult stage. Normally the $^{A}\gamma$-globin gene is not expressed in adult erythroid cells of human. The expression of $^{A}\gamma$-globin gene might be stimulated by the enhancer elements of the β-globin gene because the distance between two genes was brought in proximity by recombinant DNA technique.

B 4

トランスジェニックマウスを用いたヒト異型トランスサイレチン遺伝子の発現とその役割の解析．井本岳秋1.岩永知久1.村上龍文2.前田秀一郎2.島田和典2.山村研一1（1 熊本大・医・遺伝研，2 生化一）

EXPRESSION OF THE HUMAN SERUM TTR GENE IN TRANSGENIC MICE.

Takeaki INOMOTO 1, Tomohisa IWANAGA 1, Tatsufumi MURAKAMI 2, Syuichiro MAEDA 2, Kazunori SHIMADA 2, Ken-ichi YAMAMURA 1(1 Inst. Med. Gen. Kumamoto Univ. Med. Sch., Kumamoto; 2 Dept. Biochem. Kumamoto Univ. Med. Sch. Kumamoto)

トランスサイレチン(TTR) 遺伝子は肝、脈絡そう、卵黄のうで発現しているが、その調節機構は明らかにされていない。また、家族性アミロイドポリニューロパシー(FAP) で沈着しているアミロイドは30番目のバリンがメチオニンに置換した異型TTR であることが明らかにされている。TTR 遺伝子の発現調節とFAP の発症機構を明らかにするため、ヒト異型TTR 遺伝子(13.6 kb) を導入したトランスジェニックマウス8 匹を得た。マウス血中のヒト異型TTR 濃度をWestern 法で調べたところ、2 mg/dl から17mg/dl の濃度であった。TTR 遺伝子発現の組織特異性をNorthern法で調べたところ肝、脳および腎でmRNAが検出された。これらのマウスでアミロイドの沈着がおこるかどうか、現在経時的に追究している

B 5

EXPRESSION OF CD5 REGULATES RESPONSIVENESS OF HUMAN T CELL TO INTERLEUKIN-1. Yasuharu NISHIMURA(Dept.Genet.,Kyushu Univ.,Fukuoka) and Steven,J.,BURAKOFF(Div.Pediat.Oncol.,Dana-Farber Cancer Inst., Boston)

The role of the CD5 surface molecule in T cell responsiveness to interleukin-1 (IL-1) was examined. A $CD5^-$ mutant Jurkat cell line was generated from a $CD5^+$ parent cell line. This $CD5^-$ mutant subclone was infected with a defective retrovirus containing the CD5 cDNA and/or the neo gene encoding C418 resistance. The $CD5^+$ wild type Jurkat produced interleukin-2 (IL-2) in response to anti-CD3 monoclonal antibody (MAb), OKT3, crosslinked to a solid surface. IL-2 production was enhanced by co-culture with IL-1 or anti-CD5 MAb. Neither the $CD5^-$ mutant nor the $CD5^-$ G418-resistant infectant responded to anti-CD5 MAb or to IL-1. Responsiveness to IL-1 was restored by cell surface expression of CD5 in the $CD5^+$ infectant. The correlation of CD5 expression and specific binding of recombinant IL-1ß was examined in these cell lines. Both the specific binding (at 4^oC) and subsequent internalization (at 37^oC) of radioactive recombinant IL-1ß was equivalent in the $CD5^+$ infectant and the $CD5^+$ wild type Jurkat cell, whereas specific binding of radioactive recombinant IL-1ß was markedly decreased in the CD5 G418-resistant infectant. These observations strongly suggest that cell surface expression of CD5 regulates binding of and responsiveness to IL-1.

B 6

ACTIVATION OF HUMAN $CD8^+$T CELLS BY AN ANTIGEN SPECIFIC $CD4^+$T CELL LINE IN VITRO. Mitsuru FUKUNAGA, Kenji HIRAYAMA, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka)

We generated an antigen(SCW) specific T cell line from PBMC of a healthy donor with an intermediate response to SCW. Proliferation of the line was completely blocked by a mAb directed against HLA-DR. This line activated autologous $CD8^+$T cells to proliferate in an antigen specific manner in the presence of autologous monocytes, and this activation was mediated by a factor derived from this line, and was blocked by a mAb against HLA class I molecules. The resultant $CD8^+$T blasts showed antigen non-specific suppression on the proliferative response of autologous $CD4^+$T cells and revealed no cytolytic activity. This antigen specific activation of $CD8^+$T cells in vitro by the antigen specific $CD4^+$T cell line is expected to contribute to analyses of $CD8^+$T cell subsets, particularly in the down regulating system, at both cellular and molecular levels.

B 7

HLA AND SILICOSIS IN JAPAN. Koji HONDA, Kenji HIRAYAMA, Ikuo KIKUCHI, Akinori KIMURA, Hirotoshi SHINAGAWA and Takehiko SASAZUKI (Dept. Genet. Med. Inst. Bioreg., Kyushu Univ., Fukuoka), Nao MATSUBAYSHI, Hiroshi NAGATO and Hajime TAMAI (Dept. Psychosoma. Med. Kyushu Univ., Fukuoka)

Silicosis is a chronic fibrotic lung disease caused by prolonged inhalation of silica dust. To investigate genetic factors involved in the development of silicosis, HLA class I, classII and 4th component of complement(C4) were analysed in Japanese patients with silicosis by serological means and by restriction fragment length polymorphism(RFLP) analysis. Serological data revealed that there were increases in frequencies of HLA-A11 (relative risk(rr)=1.84, $p<0.02$), Bw54(rr=2.34, $p<0.001$), Cw1(rr=1.69, $p<0.02$), DR4(rr=1.90, $p<0.04$) and DRw53(rr=2.05, $p<0.05$), while the frequency of HLA-A24(rr=0.47, $p<12.2$) or Bw52(rr=0.23, $p<15.00$) was decreased. We also found an increase in frequency of specific C4 RFLP pattern which corresponded to C4A3B5 allotypes(rr=2.50, $p<0.02$), and a decrease of DQα and β RFLP patterns corresponding to HLA-Dw12 haplotype(rr=0.17, $p<0.02$) by RFLP analyses. These data susggested that A11-Bw54-Cw1-C4A3B5-DR4-DRw53-(DQw4) was susceptible haplotype and A24-Bw52-C--DR2-DQw1-Dw12 was resistant haplotype for silicosis.

B 8

HLA-DQ AS A MAJOR STIMULATORY MOLECULE IN AUTOLOGOUS MIXED LYMPHOCYTE REACTION(MLR). Kazuhiko FUJISAWA, Nobuhiro KAMIKAWAJI, Michio YASUNAMI, Akinori KIMURA, Kenji HIRAYAMA, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka)

In order to investgate the role of HLA classII molecules in auto or allo MLR, we generated 5 kinds of L cell transfectants expressing classII genes(DR2, DR4, DQw1, DQw4, DRw53), and analyzed the contribution of DR and DQ molecules to MLR. In primary and secondary MLR against classII transfectants and blocking experiments of MLR, it was shown that DR molecules function as dominant stimulator molecules in allo MLR, whereas DQ molecules as well as DR molecules stimulate equally auto MLR. To investigate further, we determined the clone size of MLR reactive $CD4^+$T cell utilizing the limiting dilution analysis. Frequencies of auto DR2, DR4, DQw1, and DQw4 reactive $CD4^+$T cell are estimated to be 1/5600, 1/12000, 1/1600 and 1/1300 respectively, confirming the important role of DQ molecules in auto MLR. This difference of clone sizes between DQ and DR reactive T cell in auto MLR is in reverse correlation with the expression level of DQ and DR molecules on human monocytes. Biological function of auto DQ reactive clones are now under investigation.

B 9

HLA and RFLP analysis of IDDM in Japanese.

Juan M. APARICIO, Akemi WAKISAKA, Akio TAKADA, Miki AIZAWA (Dept. Patol. Hokkaido Univ., Sapporo), Nobuo MATSUURA (Dept. Pediat., Hokkaido Univ. Sapporo)

In order to investigate the genetic factors in relation to insulin dependent diabetes mellitus (IDDM), several families and 57 unrelated patients were first HLA typed, where DQ antigens (especially DQw4) were thought to be the restriction molecule to develope the disease as in Caucasians, however, the 57th position of the DQβ chain, which has been demonstrated to be non-aspartic (non-asp-57) in Caucasians, surprisingly it was found to be aspartic acid (asp-57) in Japanese. A gene conversion like event was thought, between DQ and DR molecules, was demonstrated later not to occur, after 14 enzymes digestion within the families, where affected and healthy members shared the same HLA genotype. Therefore, IDDM susceptibility in Japanese is determined by the DRβ 57th position, while DQβ is for Caucasians. Moreover, such susceptibility genes which are associated with the DRβ molecule, were found to be inherited in a recessive trait.

B 10

COMPARATIVE MAPPING OF HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX BY PULSED FIELD GEL ELECTROPHORESIS. Katsushi TOKUNAGA (Dept. Anthrop., Univ. Tokyo, Tokyo), G. SAUERECKER, P. KAY, F. CHRISTIANSEN, R.L. DAWKINS (Dept. Clin. Immunol., Royal Perth Hosp., Perth), Tohru NAOHARA (Central Japanese Red Cross Center, Tokyo) and Takeo JUJI (Blood Transfus. Serv., Tokyo Univ. Hosp., Tokyo)

The long-range molecular map of the human major histocompatibility complex(MHC) was examined in multiple examples of various Caucasian and Japanese MHC haplotypes using pulsed field gel electrophoresis in combination with infrequently cutting restriction endonucleases. Extensive differences in restriction fragment length were observed in different MHC haplotypes. However, each MHC haplotype showed specific genomic characteristics including major deletions, duplications or insertions suggesting that these MHC haplotypes have been conserved in the human evolution. Some of the major rearrangements were consistent with the deletions or duplications previously described or suggested with conventional DNA techniques and protein typing, whilst others were newly recognized.

B 11

CONSTRUCTION OF HLA CLASS II TRANSGENIC MICE. Akinori KIMURA, Tomohisa IWANAGA, Takeshi INAMITSU, Kenji HIRAYAMA, Michio YASUNAMI, Mitsuru FUKUNAGA, Kazuhiko FUJISAWA, Yoshinori FUKUI, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. of Genetics, Med. Inst. of Bioregulation, Kyushu Univ.)

To investigate the regulation of the expression of HLA class II molecules and their roles in the regulation of immune responses, we introduced DRα , DRβ or DQα with DQβ genes into fertilized murine eggs and established several strains of transgenic mice. The DR gene was expressed properly in one DRα strain (DRα-6) while another strain (DRα-3) expressed the gene only in thymus. Because we found a correlation of the methylation and the expression of the DRα gene in various human cell lines, we investigated the methylation state of the introduced gene in these two strains and found that the DRα gene was less methylated in the former than in the latter. The DQα gene was expressed in spleen, thymus and brain, while the DQ β gene was transcribed in spleen, thymus and lung in DQ$\alpha\beta$ transgenic mouse. These data indicated that the introduced human MHC class II genes could be transcribed properly in a tissue specific manner as murine MHC class II genes in mice, and that these transgenic mice would be useful to reveal the function of individual HLA class II gene.

B 12

GENETIC CONTROL OF THE IMMUNE RESPONSE IN HLA-DQw1 TRANSGENIC MICE.
Takehiko SASAZUKI, Tomohisa IWANAGA, Michio YASUNAMI, Takeshi INAMITSU, Mitsuru FUKUNAGA, Kenji HIRAYAMA, Akinori KIMURA, Yasuharu NISHIMURA (Dept. Genet., Kyushu Univ., Fukuoka) and Katsuiku HIROKAWA (Tokyo Metropol. Inst. Gerontol., Tokyo)

In order to investigate the role of HLA-DQw1 gene in immune regulation, HLA-DQw1 transgenic B6 mice (B6-DQw1) were produced. DQw1 α and β genes were cloned from HLA-Dw12-DQw1 haplotype associated with low responsiveness to Schistosoma japonica(Sj) antigen and with high responsiveness to streptococcal cell wall(SCW) antigen in man. F1 progeny between B6-DQw1 and C3H mice were immunized with L cells expressing HLA-DQw1 gene. DQw1 negative F1 mice produced antibody directed against DQw1, whereas DQw1 positive mice did not indicating that transgenic mice acquired the tolerance to HLA-DQw1. Mice were immunized with soluble antigens and proliferative response of lymphnode T cells to antigens was investigated. B6 mice did not respond to SCW antigen but did respond to Sj antigen. On the otherhand, B6-DQw1 mice showed strong immune response to SCW antigen and it was inhibited by anti DQw1 or anti L3T4 monoclonal antibody. About 30% of B6-DQw1 mice did not respond to Sj antigen. These observations indicate that HLA-DQw1 molecules expressed in transgenic mice are functional and that HLA-DQw1 gene controlls the immune responsiveness in these mice.

B 13

遺伝性高フェニルアラニン血症の病型診断と頻度に関する研究・谷本正志・大和田操・北川照男（日本大学医学部小児科）・STUDY ON THE INCIDENCE OF HYPERPHENYLALANINEMIC SYNDROME. Masashi TANIMOTO, Misao OWADA, Teruo KITAGAWA. Dept. Pediatrics. Nihon Univ. School of Medicine. 1-8-13 Kandasurugadai Chiyoda-Ku Tokyo. 101

自験例32例の遺伝性高フェニルアラニン（Phe）血症の病型診断を行い，その成績を我が国の新生児マス・スクリーニングで発見された症例の病型別頻度と比較するとともに，諸外国の成績と比較検討した。即ち持続的な高Phe血症を示す症例32例に対し，①テトラヒドロビオプテリン（BH_4）負荷試験，②尿中プテリン化合物の分析，③赤血球ジヒドロプテリジン還元酵素（DHPR）活性を測定して，フェニルケトン尿症（PKU），良性高Phe血症，BH_4欠乏症（DHPR欠損症およびPTPS欠損症）に分類した。その結果，22例がPKU，6例が良性高Phe血症，4例がBH_4欠乏症に分類され遺伝性高Phe血症32例中BH_4欠乏症は4例で，全体の12.5％を占めていた。我が国ではPKUのマス・スクリーニングにより10年間にPKU 116例，良性高Phe血症45例，BH_4欠乏症11例が発見されており，BH_4欠乏症は全体の6.5％，PKUに対しては約1/10の発生で，欧米における1～3％との報告に比べてその頻度が高い。しかし我が国のPKUの頻度は出生11万人に1人と欧米よりも著しく低いためにBH_4欠乏症の頻度が見かけ上高いのであり，絶対的な発生頻度には差がないと考えられる。但し，我が国ではPTPS欠損症が大部分を占める一方，欧米ではDHPR欠乏症が約40％であり，各々の病型の発生頻度には人種差が認められていた。

B 14

TWO VARIANT CASES OF PEROXISOMAL DISORDERS: ZELLWEGER-LIKE SYNDROME AND 3-KETOACYL-CoA THIOLASE DEFICIENCY

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We investigated two patients with clinical findings similar to those of Zellweger syndrome, and it was elucidated that they were variant forms of peroxisomal disorders. [CASE 1] A baby boy who died at age 5 months was analyzed. Accumulation of very long chain fatty acids and intermediates of bile acid synthesis, defects in the enzyme proteins of peroxisomal ß-oxidation and Dihydroxyacetone phosphate acyltransferase (DHAP-AT) were noted. However, immunocytochemical study revealed that hepatic peroxisomes were present at a level similar to that in the control. These observations suggest the presence of a further heterogeneity in Zellweger syndrome and that the pathogenesis in this variant case is different.
[CASE 2] A 5 month old girl with hypotonia, convulsions, psychomotor retardation and hepatomegaly was investigated. Very long chain fatty acids in serum sphingomyelin were increased, and ^{14}C-lignoceric acid oxidation in fibroblasts was severely decreased. Hepatic peroxisomes were apparently normal. Measurement of individual enzyme activity revealed that peroxisomal 3-ketoacyl-CoA thiolase was deficient. Amount of enzyme protein, biosynthesis and intracellular localization of this enzyme was normal. This case is apparently a heterogeneous type of peroxisomal 3-ketoacyl-CoA thiolase deficiency.

B 15

STUDY OF THREE PATIENTS WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY: DETECTION OF ONE NOVEL MISSENSE AND ONE NONSENSE MUTATIONS.

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Ornithine transcarbamylase (OTC) is a nuclear DNA coded mitochondrial protein, and its deficiency is the most common inborn error of the urea cycle. We already reported the structural organization of the entire human OTC gene, which is about 73 kb long and contains 10 exons. In the present study, we define the molecular basis of three patients. Study of two unrelated male patients showing abnormal Km values of OTC revealed that they have the same one base substitution within exon 8, which causes a novel missense mutation. In the case of a female patient, we found by applying a direct genomic sequencing method of amplified DNA that one of the OTC alleles carries a C-to-T substitution in a TaqI site of exon 5, and also found that this substitution results in a nonsense mutation.

B 17

Molecular Genetics of Gaucher's Disease: Correlation of mutations in Glucocerebrosidase Gene with Clinical Phenotypes. Shoji Tsuji (Dept. Neurol., Brain Res. inst., Niigata Univ., Niigata), Brian M. Martin and Edward I. Ginns (Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda)

Gaucher's disease, the most common sphingolipidosis, is caused by a deficiency of lysosomal glucocerebrosidase (E.C.3,2,1,45). On the basis of clinical signs and symptoms, three major clinical phenotypes have been identified. In order to identify mutations in each phenotype, glucocerebrosidase genes were cloned from patients with types 1 and 2. Comparison of sequences of exons, splice junctions, and flanking regions revealed a single base substitution (T to C) in exon 10 of type 2 glucocerebrosidase gene. This mutation was shown to be highly frequent among neuronopathic Gaucher's disease. On the other hand in type 1 glucocerebrosidase gene, we identified a single base mutation (A to G) in exon 9. Allele-specific hybridization study employing oligonucleotide probes with high specific radioactivity showed that the mutation was exclusively found in type 1 Gaucher's disease.

B 18

成人型GM1ガングリオシドーシス1家系の考察．得田彰・平山幹生・武藤多津郎・清澤和弘・中崎繁明・萩野正樹・中永昌夫・藤木典生．（福井医大・第二内科）．
A FAMILY OF ADULT GM1-GANGLIOSIDOSIS. Akira TOKUDA, Mikio HIRAYAMA, Tatsuro MUTOH, Kazuhiro KIYOSAWA, Sigeaki NAKAZAKI, Masaki HAGINO, Masao NAKANAGA, and Norio FUJIKI (2nd Dept. Int. Med., Fukui Med. Sch., Fukui)

１０歳頃構音障害で発症し、ジストニアを主徴とする錐体外路症状、角膜の混濁、腰椎の扁平化、リンパ球の空胞化を呈した２５歳の男性において、リンパ球、血漿、培養皮膚線維芽細胞の全てにおいて、人工基質や、GM１ガングリオシド、asialofetuin，に対するβ－ガラクトシダーゼ活性の著明な低下を認めた。また、両親は、いとこ婚であり、両親と父方の祖父において、酸性β－ガラクトシダーゼ活性は、正常者の約半分を呈した。これらのことから、常染色体劣性遺伝形式をとるβ－ガラクトシダーゼ欠損症と考えられた。β－ガラクトシダーゼ活性低下をみる遺伝性疾患は種々あるが、シアリダーゼを含む他のライソゾーム水解酵素は正常であることや、ジストニアと構音障害を中心とする症状と骨変化があり、ガルゴイル様顔貌、肝脾腫、Cherry-red spot、小脳症状等がないことより、GM１ガングリオシドーシス、中でも非常に希な成人型GM１ガングリオシドーシスと診断してよいと思われた。

B 19

肝型糖原病42例の病型に関する研究・岩本孝夫・大和田操・北川照男（日本大学医学部小児科）・STUDY ON 42 CASES WITH HEPATIC GLYCOGENOSIS. Takao IWAMOTO, Misao OWADA, Teruo KITAGAWA. Dept. Pediatrics. Nihon Univ. School of Medicine. 1-8-13, Kandasurugadai, Chiyoda-Ku Tokyo. 101

肝腫大，低身長，低血糖発作などの症状を認め肝型糖原病が疑われた42例に対して，肝，筋，血球成分を用いてグリコーゲン代謝関連酵素の検索およびグリコーゲンの定量を行うとともに，Fernandesらによって提唱された負荷試験による肝型糖原病のスクリーニング法を施行して病型を決定し，その成績を我が国および諸外国から報告されている肝型糖原病の疫学調査結果と比較した。自験例42例の病型別内訳は，Ⅰ型18例（Ia 17, Ib 1），Ⅲ型９例，Ⅳ型１例，Ⅵ型１例，Ⅷ型11例，Ⅸ型１例および病型が確定できなかった症例が１例であり，Ⅰ型が43％と全例の約半分を占め，Ⅷ型とⅢ型がそれぞれ26％と21％であった。この成績は1983年に厚生省心身障害研究班が行った代謝性蓄積症に関する全国調査において報告された肝型糖原病121例における病型分布とほぼ一致していたが，我が国で1960年代および1970年代に行われた２回の調査成績とは明らかに異っていた。即ち，Ⅰ型の占める率は前者で98％，後者で74％と報告されており，漸次Ⅰ型が減少しているように見えるが，これは診断技術の進歩によって，Ⅰ型と類似の症状を示すⅢ，Ⅵ，Ⅷ型などの病型の診断が可能になったためと結論される。欧米の報告では，イスラエル，ノルウェー，イギリスなどではⅢ型が圧倒的に多いのに比べて，西独の報告は我が国に似た分布を示しており，肝型糖原病の各病型の遺伝子頻度には人種差が認められることが示唆された。

B 20

CHARACTERIZATION OF HUMAN RESIDUAL CATALASE OF A JAPANESE ACATALASEMIC PATIENT BY ISOELECTRIC FOCUSING AND SDS-PAGE USING BLOTTING TECHNIQUES.
Kazuhiko SUZUKI, Yukinori SATOH and Masana OGATA
(Dept.of Public Health, Okayama Univ.Med.School, Okayama)

Agarose gel isoelectric focusing and immunoenzymatic reaction after electroblotting of acatalasemic residual catalase, normal catalase and mixture of both catalases was performed using erythrocyte C fraction.

Results indicated that isoelectric point of acatalasemic residual catalase was identical with that of normal catalase.

Subunit sizes of acatalasemic residual and normal catalases in the C fractions of erythrocytes were also identical as revealed by SDS-PAGE, followed by electroblotting and immunoenzymatic amplification.

The results indicated no substantial difference in molecular charge and size of catalase protein between acatalasemic and normal Japanese.

B 21

ACID AND ALKALINE STABILITIES OF CATALASE IN THE ERYTHROCYTES OF ANEMIC ACATALASEMIC MICE.

Junichi FUJIMURA and Masana OGATA
(Dept. of Public Health, Okayama Univ. Med. School, Okayama)

Acid and alkaline stabilities of catalase in anemic and none-anemic mouse bloods were examined with crude catalase solution prepared by DEAE column.
1. Acid and alkaline stabilities of blood catalase of anemic or none-anemic acatalasemic mice were lower than those of normal or heterozygous hypocatalasemic mice.
2. Catalase in anemic blood was more stable for acid and alkaline than that in none-anemic blood of acatalasemic mice.

B 22

Clinico-genetical Studies On Idiopathic Familial Basal Ganglia Calcification.

Kenjiro MASUDA, Hisaomi KAWAI, Yoshihiko NISHIDA, Toshihiko SEBE, Koichi SATO, Takako NARUO and Shiro SAITO (First Dept. of Int. Med., The Univ. Tokushima, Tokushima)

We reported a family of idiopathic familial basal ganglia calcification(IFBGC), and reviewed the disease on its clinical and genetical features. The BGC patients due to parathyroid disorders or found in infancy were excluded. The proband of our family was a 16-year-old girl whose parents were not consanguineous. Since the age of 12 she has complained of pulsative headache and had cerebellar ataxia. Both her mother and maternal grandfather had BGC but they showed no symptoms. There have been reported 48 patients of 8 families who were found through 2 to 3 generations. The age when BGC cases was found ranged from 7 to 78 years old. The BGC patients consisted of 26 males and 22 females, beeing the sex ratio nearly 1:1.

Neurological symptoms, such as mental deterioration, speech disturbance, extrapyramidal signs and cerebellar ataxia, were observed in half of these cases. Penetrance was calculated as 100% and segregation ratio 0.5. These data suggest that FIBGC is inherited as an autosomal dominant trait with high penetrance rate.

B 23

APERT SYNDROME WITH POLYSYNDACTYLY.

Yoshinori IZUMIKAWA, Kenji NARITOMI, Shoko IKEMA, Yoshinobu GOYA, Noboru SHIROMA and Kiyotake HIRAYAMA (Dept. Pediatr., Univ. the Ryukyus, Okinawa)

The patient, a newborn girl, was born to healthy and unrelated parents, both 32 years of age. She was delivered by cesarean section at 36 gestational weeks. Birth weight was 3024g, length 50.0cm and OFC 34.5cm. Examination revealed asymmetric face with acrocephaly, large fontanel, downslanting of palpebral fissures, prominent eyes, shallow orbits, beaked nose, narrow palate and bifid uvula. The hands showed broad thumbs and syndactyly of 2-5 fingers. The feet showed syndactyly of all digits with broad great toes. Typical Apert syndrome was suspected. However, roentgenological examinations revealed partial proximal bifid of duplicated 1st metatarsals.

Seemingly typical Apert syndrome with polysyndactyly have been reported by Maroteaux and Fonfria (1987). They suggested a possibility of a distinct syndrome separated from Apert syndrome. The present patient seemed to similar to the cases reported by them. Distal duplication of halluces have been reported in the families with Saethre-Chotzen syndrome (Robinow-Sorauf syndrome, Carter et al. 1982). Thus, there is a possibility that Apert syndrome with polysyndactyly might be a variant form (Maroteaux-Fonfria syndrome).

B 24

APOLIPOPROTEIN E ALLELES AND HYPERLIPOPROTEINEMIA

Masaaki ETO, Kiyoshi WATANABE, Kaneo ISHII, Isao MAKINO
(Dept of Internal Med. 2, Asahikawa Medical College, Asahikawa, Japan)

There is growing evidence that the apo E alleles may be associated with hyperlipoproteinemia (HLP). The homozygote, apo E2/2 is often seen in type III HLP. A strong association of the ε4 allele with hypercholesterolemia has been suggested. In this study, we have examined the apo E allele frequenices in normolipidemia (n=129), non-familial hypercholesterolemic (FH) type IIa HLP (n=40), non-FH type IIb HLP (n=35), type III HLP (n=17), type IV HLP (n=59), type V HLP (n=19) and heterozygous FH (n=51) in Japan, and compared these frequencies between normolipidemia and hyperlipoproteinemia. All subjects were unrelated to one another, and had normal renal, hepatic and thyroid functions. The frequency of the ε4 allele was significantly higher in type IIa(18.7%), IIb(21.4%) and V(29.0%) HLP and FH(16.6%) than in normolipidemia(8.9%), whereas the frequency of the ε2 allele was significantly higher in type III(70.6%) and IV(11.0%) HLP than in normolipidemia(3.1%). These results suggest that the ε4 allele is associated with non-FH hypercholesterolemia (type IIa and IIb HLP), type V HLP and FH, whereas the ε2 allele is associated with type IV HLP as well as type III HLP.

B 25

ANALYSIS OF THE APOLIPOPROTEIN AI GENE IN A PATIENT WITH APOLIPOPROTEIN AI DEFICIENCY. Naoko HATTORI[1], Yoshikazu HIASA, Kimiko YAMAKAWA[1], Isao TANAKA[3], Hisako YANAGI[1], Tomoyuki MATSUNAGA, Kenji YUZAWA[1] and Hideo HAMAGUCHI[1] ([1]Dept. Hum. Genet., Univ. Tsukuba, Tsukuba; [2]Dept. Cardiol., Komatsushima Red Cross Hosp., Komatsushima; [3]Eisai Tsukuba Research Labolatories, Tsukuba)

To define a mutant gene which causes hereditary high density lipoprotein (HDL) deficiency, we analyzed the gene of apolipoprotein AI (apoAI), the major protein constituent of HDL, in a female patient with apoAI deficiency associated with a marked reduction of HDL cholesterol and coronary heart disease. No gross abnormality in the apoAI and CIII gene region of the patient was detected by Southern blot analysis using apoAI genomic DNA probe. However, she was homozygous for a RFLP haplotype in this gene region. Her parents were first cousins and the probability of occurrence of the homozygosity for the RFLP haplotype by chance was estimated to be less than 0.024 in offspring from a first-cousin marriage. These data suggest that the patient is homozygous for a mutant apoAI gene resulting in a severe deficiency of apoAI. To elucidate the mutation of this gene, we have constructed genomic DNA library from her DNA. Some positive clones were isolated from 3.2×10^5 recombinants and at least one of them has been confirmed to include the apoAI gene coding region by Southern hybridization. We are now sequencing the cloned gene.

B 26

ANALYSIS OF A MUTANT LDL RECEPTOR GENE ASSOCIATED WITH A TaqI 1.5kb VARIANT BAND. Takaaki OKAFUJI, Kimiko YAMAKAWA, Hisako YANAGI, and Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Tsukuba), Yukio IWAMURA (Dept. Microb., Univ. Tsukuba, Tsukuba) and Isao TANAKA (Eisai Co., Ltd., Tsukuba Reserch Laboratories, Tsukuba)

The recognition sequence for TaqI contains the CpG dimer, which is the major site of methylation of human DNA. CpG dimer is probably a mutation hot spot. Mutations in the gene for LDL receptor give rise to familial hypercholesterolemia (FH). To study point mutations detected with TaqI in the LDL receptor gene in Japanese, we have analyzed DNA samples from 14 FH families with TaqI and the Southern blot hybridization using a LDL receptor cDNA probe. A variant TaqI 1.5kb band associated with the mutant LDL receptor gene was detected in one of 14 families. This TaqI variant band has not been detected in 82 unrelated healthy Japanese. Analysis of the mutant LDL receptor gene using various LDL receptor cDNA fragment probes and restriction enzymes revealed that a missing TaqI site is located within an intron, about 340 base pair (bp) 5' to exon 7. We are now cloning the DNA containing the missing TaqI site to determine whether this mutation is directly responsible for the desease in the family.

B 27

ANALYSIS OF MUTATIONS IN THE LDL RECEPTOR GENE AMONG JAPANESE PEDIGREES WITH FAMILIAL HYPERCHOLESTEROLEMIA

Kimiko YAMAKAWA[1], Hisako YANAGI[1], Takaaki OKAFUJI[1], Yukio IWAMURA[1], Kenji YUZAWA[2], Yae SHIMAKURA[2], Koichi KAWAI[2], Shigeru TSUCHIYA[3] and Hideo HAMAGUCHI[1] ([1]Dept. Hum. Genet., Inst. Basic Med. Sci., [2]Inst. Clin. Med., [3]Inst. Comm. Med., Univ. Tsukuba, Tsukuba)

Mutations in the gene for LDL receptor give rise to familial hypercholesterolemia (FH). To characterize the mutations in the LDL receptor gene and to examine genetic heterogeneity of FH in Japanese at the DNA level, we have analyzed the mutant LDL receptor genes in 14 typical heterozygous FH families by the Southern blot hybridization using the LDL receptor cDNA clone as a probe. Four different partial deletions were detected, suggesting that deletions may not be rare in the mutant genes in typical FH. In the remaining ten mutant LDL receptor genes, no gross alteration in the gene has been identified. For the ten mutant genes, we determined the RFLP haplotypes of the LDL receptor gene, using seven RFLPs detectable in the gene. Seven haplotypes were observed in the ten mutant LDL receptor genes. There are no predominantly occurring unique haplotypes which can characterize the FH gene in Japanese. These data suggest the presence of preferential sites for major rearrangements resulting in deletions within the LDL receptor gene. The results also indicate considerable genetic heterogeneity in FH in Japanese.

B 28

A TRIAL TO DIFFERENTIATE FAMILIAL COMBINED HYPERLIPIDEMIA FROM FAMILIAL HYPERCHOLESTEROLEMIA WITH RFLPs OF LDL RECEPTOR GENE.

Hisako YANAGI[1], Kimiko YAMAKAWA[1], Takaaki OKAFUJI[1], Kenji YUZAWA[2], Kouichi KAWAI[2], Yae SHIMAKURA[2], Shigeru TSUCHIYA[3] and Hideo HAMAGUCHI[1] ([1]Dept. Hum Genet., Inst. Basic Med. Sci., [2]Inst. Clin. Med., [3]Inst. Comm. Med., Univ. Tsukuba, Tsukuba)

Familial combined hyperlipidemia (FCHL) is one of the commonest autosomal dominant disorder caused by unkown mechanism. It is considered that the frequency of this disease is higher than that of familial hypercholesterolemia (FH), which is caused by the deficiency of LDL receptor. The diagnosis of FCHL, however, is not easy because there is no diagnostic procedure except family studies, and because the lipid level of the affected individual very with time. Especially the diffrential diagnosis between FCHL and FH is important but difficult in some hypercholesterolemic families. To confirm the diagnosis we examine the RFLPs of LDL receptor gene of the hypercholesterolemic families that show autosominal dominant inheritance, and verify whether hypercholesterolemia is linked to the RFLPs haplotype or not. The results show that there are some autosomal dominant hypercholesterolemic families that are not linked to the LDL receptor gene, and some of them have clinical features of FCHL. This meathod is useful for the differential diagnosis between FCHL and FH.

B 29

ZYGOSITY DIAGNOSIS OF TWINS BY MENDELIAN TRAITS. Syuichi OOKI, Akio ASAKA (Sch.Health Sci.,Fac Med., Tokyo Univ., Tokyo)

This paper deals with the method of estimating the probability of being MZ, when many Mendelian traits are concordant. The formula of the concordance probability is calculated by twins' phenotype and parents' phenotype whether they are known or unknown. The procedures are applicable to all sorts of Mendelian traits, provided the mode of inheritance is clear. The value of the concordance probability can be given by using the gene frequency among Japanese general population. We made several tables of these values, for example ABO, MN, Rh blood type and so on, for practical use. We can calculate the probability of being MZ by using these value. At present, many Mendelian traits can be examined easily under the reasonable price. We concluded that diagnosis of zygosity by Mendelian traits can be used practically.

B 30

ZYGOSITY DIAGNOSIS OF TWINS BY QUESTIONNAIRE. Syuichi OOKI, Akio ASAKA (Sch.Health Sci.,Fac Med., Tokyo Univ., TOKYO) Kazuaki YAMADA (Dept. Pub.Health., Sch.Med., Showa Univ., Tokyo)

Subjects were 189 twin pairs, consisting of 165 MZ and 24 DZ who entered the junior high school affiliated to Tokyo University, and a part of their mothers (44 MZ's and 9 DZ's). Their zygosity were previously diagnosed by many genetic markers. We tried to diagnose their zygosity by questionnaire. Questionnaire included three questions. The first question was the similarity of twins: "How are you alike ?". The second was the frequency of being mistaken: "How often are you mistaken ?". And the third was the person: "By whom are you mistaken ?". According to the degree, 1-3 points, 1-3 points, and 1-4 points was given for each question. And the sum of the points of each pair of twins or their mothers was calculated. Zygosity was diagnosed by the sum of points. The result was that most twins were diagnosed correctly by use of adequate cutting point and the answers of twins' mother were more effective for zygosity diagnosis. We concluded that zygosity diagnosis by questionnaire was convenient and useful,in particular,for epidemiological research.

B 31

SCHOLASTIC ABILITIES IN TWINS. Akio ASAKA, Shuichi OOKI, Yoshihiro NAGAI (Dept. Mental Health, Tokyo University, Junior High School affil. to Tokyo Univ., Tokyo)

Subjects were 127 pairs of twins, consisting of 100 monozygotic twins (MZ) and 27 dizygotic twins (DZ), who had passed the entrance examination of the junior high school affiliated to Tokyo University in the last 8 years. Items analysed were the results of entrance examination, one of which (exam I) included mainly Japanese, and the other (exam II) mainly mathematics. And also available were standard ability test which were done soon after students' entrance, consisting of Japanese, social science, mathematics and natural science. After the standardization (mean=0 SD=1) of scores according to sex and each item, the intraclass correlation coefficient were calculated. The intraclass correlation coefficients, .5810, .4976, .5639, 6588, .5332 and .6374 in the above order, were significantly higher than zero among all items for MZ, but nothing for DZ (.2520, .1820, .0067, .1228, .2215 and .3516),suggesting genetic influence on scholastic ability. The value was higher in exam II than in exam I in MZ. With respect to standard ability test the most highest value was seen in mathematics for MZ. These findings indicate the presence of environmental factors effecting the scholastic ability of mathematics, which were already confirmed by our previous study.

B 32

CONJOINED TWINS IN JAPAN, 1979-1985. Yoko IMAIZUMI (Inst. Pop. Prob., Ministry of Health and Wealfare, Tokyo)

Nation-wide data in Japan on the 112 sets of conjoined twins from fetal deaths and from postnatal deaths during 1979-1985 were analysed. Female conjoined twins accounted for 60% of cases. The incidence rate of conjoined twins remained constant except in 1985. Overall incidence rate was 10 per million births. Maternal age effect was found in mothers over the age of 40, where the highest incidence rate was obtained. The incidence rate of conjoined twins increased with birth order. There was no seasonal variation in the time of conception.

B 33

TWIN STUDY ON HERITABILITY FOR SERUM LIPIDS AND CHOLESTEROL IN LATER ADULTHOOD. Kazuo HAYAKAWA (Dept. Publ. Health, Kinki Univ., Osaka)

A twin study was conducted on serum concentrations of lipids and cholesterol. The subjects were 107 pairs of adult twins (81 monozygotic, 26 dizygotic), who were residing in the community and aged over 50. The serum items examined in this study were total cholesterol, HDL cholesterol, LDL cholesterol, trygliceride, β-lipoprotein, phospholipid, free fatty acid, apolipoproteins (A-I, A-II, B, C-II, C-III, E). The heritability was estimated from the data of male twins through variance analysis. Environmental variance was statistically adjusted between monozygotic and dizygotic twins through multiregression analysis. Strong heritability was shown in HDL cholesterol (0.667), apo B (0.667), LDL cholesterol (0.608). On the other hand, triglyceride, apo A-II, and apo C-III showed a low level of heritability (0.2 and less).

B 34

A case of monozygotic twin reared apart with atypical schizophrenic mother. Tooru ISHIDA, Hiroshi YONEDA, Masato YOKOI, Youko NAGAI, Takako OZAKI, Hiroyuki ASABA and Toshiaki SAKAI. Dept. Neuropsyciat., Osaka Medical College, Osaka

We report here a case of 10 year-old male monozygotic twins reared apart, whose mother is diagnosed as atypical schizophrenia. One of the twins was reared by his atypical schizophrenic mother and co-twin was by his paternal grandmother since birth, after their parents divorced. When twins were 6 year-old, their parents remarried; children were brought-up together by parents thereafter. Zygosity diagnosis was made by 34 blood and serum types. In the family of this case, we found three atypical schizophrenic patients including their mother and one who committed suicide in the maternal side, and therefore this case was considered as high risk children for atypical schizophrenia. We examined this case by MRI, P300 and psychological tests. The results of MRI, P300 and IQ were very similar between the twins. However, the child reared by mother showed a lack of empathic understanding and a stereotyped interpersonal relationship to other people; these patterns were presumably learned from his atypical schizophrenic mother.

B 35

CYTOGENETIC STUDIES ON THE FEMALE PATIENTS WITH MUSCULAR DYSTROPHY.
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When we presumed the genetic mechanism of the occurrence of the female patients with X-linked Duchenne muscular dystrophy(DMD), the chromosome abnormality of the X was thought to have a biological significance on it. For this reason, we have done the cytogenetic investigations on the 22 female patients with muscular dystrophy.

The results showed that (1) four out of 13 patients of sporadic cases were carriers of the X-autosomal translocations and (2) two out of 9 patients of familial cases had 45,X/46,XX mosaicism in their lymphocytes. These two patients showed the mild symptoms of muscular dystrophy but no indication of Turner's syndrome.

These results suggested that the reciprocal translocations involving the X chromosome in the sporadic cases and the numerical abnormality of the X in the familial cases were both associated with the occurrence of the female DMD.

B 36

HETEROMORPHISMS ON RsaI-DIGESTED CHROMOSOMES: PARENTAL ORIGIN OF TRIPLO X IN A FEMALE DMD PATIENT.
Kyohko ABE*, Naoki HARADA (Kyushu Med. Sci., Cytogenet. Dept., Fukuoka), Shigeto SUGINO (Dept. Child Develop., Kumamoto Univ.), Norio NIIKAWA (Dept. Hum Genet., Nagasaki Univ., Nagasaki)

C band-like heteromorphisms on the chromosomes after digestion by RsaI or AluI were reported by Babu et al.(1988), but the detailed data have not been clear. We studied such heteromorphisms to know the constancy within a person and to confirm the co-dominant transmission from parent to child. Chromosomes from a female DMD with triplo X and her parents were digested by 200 U/ml RsaI at 37 C for 3-24 hrs and stained with Giemsa. G- and C-like bands appeared simultaneously after digestion. Though the sizes of C-like band blocks with CBG-banding did not distinctly differ among three X chromosomes, those of C-like RsaI bands varied to a certain degree. According to their sizes, we traced the transmission mode of XXX chromosomes of the patient from the parents. Of the three, one came from the father and two were transmitted from one of two X chromosomes of the mother. The result is consistent with that by the molecular study with ERT87-30. The constancy and the co-dominant inheritance were confirmed in the other family. Therefore, the RsaI heteromorphisms are new genetic markers.

B 37

INHERITANCE MODE OF FEMALE DMD-LIKE MYOPATHY: MOLECULAR AND IMMUNO-HISTOCHEMICAL STUDY. Shigeto SUGINO*, Teruhisa MIIKE (Dept. Child Develop., Kumamoto Univ.), Makoto Uchino (1st Dept. Int. Med., Kumamoto Univ.), Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ.), Ikuya NONAKA (Natl. Inst. Neurosci., Tokyo)

There is controversy on the inheritance of Duchenne muscular dystrophy (DMD)-like disease in females:autosomal recessive vs. X-linked. In order to know its inheritance mode, the DMD gene of 7 such female patients was studied using the DMD-cDNA and several X-linked sequences as probes. If available, muscle biopsy specimens were studied immunohistochemically with anti-dystrophin antibody (ADA). In a case of XXX, the copy number of the sequence ERT87-30 was one, indicating the DNA within the DMD gene is missing in 2 of her 3 X chromosomes. A one copy density for the cDMD1-9 was detected in an XX case. There was no DNA deletion in the other 5 XX cases. In one family where an XX proposita and her XY brother were both affected, segregation analysis between cDMD8/PstI-RFLPs and the disease revealed evidence of X-linked inheritance for the disease in this family. Her ADA-positive muscle cells showed mosaic distribution. In another sporadic XX case with parental consanguinity, an examination with ADA on the muscle showed positive cells, but the western-blotting with ADA revealed weak expression of the DMD gene. No informative results were obtained from the molecular study in the remaining 3 sporadic XX cases.

B 38

PFG ELECTROPHORESIS OF DNA FRAGMENTS IN AN ATYPICAL PROGRESSIVE MUSCULAR DYSTROPHY PATIENT WITH DELETION FOR PROBE 754.
Michie Shimmoto, Rei-Chen Yang, Akihiko Oshima, Yoshiyuki Suzuki (Div. Inher. Metab. Dis., Natl. Inst. Neurosci., N.C.N.P., Tokyo) Yoshiko Nomura, Masaya Segawa (Segawa Neurol. Clinic Children, Tokyo)

Among the DMD families for linkage analysis, we found out a deletion for the probe 754 in a family with atypical progressive muscular dystrophy. The DNA structure at the DMD locus in this family was estimated by Southern blot and PFG electrophoresis analysis using DMD cDNA and pobes which are located in or flanking the DMD locus. Southern blot analysis using DMD cDNA probes exhibited the same band pattern for their Hind III or Eco RI fragments, as compared to control DNA fragments. PFG electeophoresis analysis exhibited no band in the patient for the probe 754 as expected. An abnormal 700 Kb band was detected in the patient and his mother for DMD cDNA 57666 and XJ1.1 by digestion of Sfi I, whereas his other reletives and unrelated healthy Japanese examined in this study exhibited a 850 Kb band. These results indicate that this deletion endpoint lies within 700 Kb of Sfi 1 fragment revealed by DMD cDNA 57666 and the probe XJ1.1.

B 39

MOLECULAR STUDIES OF DUCHENNE MUSCULAR DYSTROPHY (DMD): A PROTOCOL PROPOSED FOR PRENATAL AND CARRIER DIAGNOSIS WITH DNA ANALYSIS. Satoshi HUJISHITA* (Dept. Neurol., Kawatana Natl. Hosp., Kawatana), Shigeto SUGINO (Dept. Child Develop., Kumamoto Univ., Kumamoto), Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki)

Prenatal or carrier diagnosis of DMD is made with molecular analysis of the genomic DNA using the DMD-cDNA and/or its linked DNAs as probes. The former is suitable for direct detection of gene deletion and the latter is generally used for segregation analysis with their RFLPs. However, to avoid misdiagnosis due to recombination, such a segregation analysis needs at least 3 probes which are distal to, proximal to and within the DMD gene. We studied 31 DMD and 2 BMD patients, their relatives and 60 normal females with Southern analysis to know the frequency of gene deletion and that of RFLPs in the Japanese, and to make a diagnostic protocol. With probes cDMD1-14, 37% of DMD/BMD patients had a deletion. The most frequent deletions were detected when using cDMD2 and cDMD8. The RFLP study revealed that the frequency of heterozygotes in every of the 3 probes was 39.6% with 99% confidence. If we combine this figure with the deletion frequency, a diagnostic rate for DMD would be 60%. We would like to present our protocol for molecular diagnosis of DMD and BMD.

B 40

APPROACH TO PRENATAL DIAGNOSIS OF FUKUYAMA CONGENITAL MUSCULAR DYSTROPHY WITH FETAL BLOOD SAMPLING.

Tadashi IIDA, Kaoru SUZUMORI, Yoshiaki YAGAMI (Dept. Obstet. & Gynecol. Nagoya City Univ. Med. Sch., Nagoya), Tatsuya ISHIKAWA (Dept. Pediat. Nagoya City Univ. Med. Sch., Nagoya) and Kuniyasu TAKADA (Div. Neuropathol., Inst. Neurobiol. Science, Tottori Univ. Tottori)

The Fukuyama congenital muscular dystrophy(FCDM) is characterized by the degeneration of skeletal muscle and the dysplasia of central nervous system which become clinically evident by the age of nine-months-old. The common symptoms are hypotonic, hypokinesia and severe mental retardation. Patient has a face with distinctive feature and severe motor disturbance. In six cases which are at risk of FCMD, serum creatinine phosphokinase activity in fetal blood were analyzed in mid-trimester of pregnancy between 19 and 22 weeks. In one case the CPK activity was remarkably high compared with the values in control group, and this fetus was confirmed to be affected with FCMD by the cerebral pathological findings obtained by necropsy. In other five case, however, CPK activities were within normal range, so their pregnancies were allowed to continue to term. In two cases, the babies were born and showed no pathological changes. Other three cases are now ongoing their pregnancies.

B 41

LINKAGE ANALYSIS BETWEEN THE JAPANESE MYOTONIC MUSCULAR DYSTROPHY LOCUS AND THE DNA MARKERS ON CHROMOSOME 19. Yuji TAKEMOTO[1], Tetsuro MIKI[1], Jun NAKURA[1], Koujin KAMINO[1], Shunichi TAKEDA[3], Kogo KUZE[3], Shin-ichiro TAKAI[2], Yuichi KUMAHARA[1], Tetsuya TAKAYANAGI[5], Kyoko SAITA[6], Mitsuhiro OSAME[4] and Tasuku HONJO[3] ([1]Dept. Med. Geriat.; [2]2nd Dept. Surg., Osaka Univ., Osaka,; [3]Dept. Med. Chem., Kyoto Univ., Kyoto,; [4]3rd Dep. Int. Med., Kagoshima Univ. Kagoshima,; [5]Dept. Neur. Nara Med. Unv., Nara,; [6]Utano Hosp., Kyoto)

Myotonic muscular dystrophy (DM) is an autosomal dominant disorder with an incidence of 1/10,000-20,000. It has been reported that the gene responsible for Caucasian DM is tightly linked to APOC2 and D19S19 which are localized to the centromere region of chromosome 19. In order to exclude the possibility that there is a genetic heterogenity among different races, we have carried out linkage analysis between the Japanese DM locus and the two DNA markers, APOC2 and D19S19. In 13 Japanese families no unequivocal crossovers were observed. Lod scores were calculated using the computer program LINKAGE (Lathrop,1984) with an age-dependent penetrance. APOC2 gives a maximum lod score of 1.41 at 0% recombination. Since the DM is linked to D19S19 with a maximum lod score of 2.14 at 0 % recombination, the Japanese DM locus might be also in the centromere region of chromosome 19.

B 42

DETECTION OF MUTATIONS IN RNA:DNA DUPLEXES. Report 2:Application of Denaturing Gradient Gel Electrophoresis(DGGE). Keiko Hiyama, Norio Takahashi, Mieko Kodaira, Chiyoko Satoh (Dept. Genet., RERF, Hiroshima)

We have confirmed that "RNase cleavage at mismatches in RNA:DNA duplexes" methodology detects substitutions, deletions, and insertions in various DNA segments from various human ß-globin genes of a maximum length of 770 bp (Report 1). In order to increase the efficiency of detection, we have applied the Lerman method of DGGE of DNA:DNA heteroduplexes to RNA:DNA duplexes. Using cloned normal and 3 thalassemic human ß-globin genes, production of ^{32}P-labeled RNA probes and hybridization of the probes with DNA samples were carried out in accord with the conditions described in the Report 1.

Eight kinds of single base mismatches, two of which were barely detected by the RNase cleavage method, deletions, and insertions of 1,4,5, and 10 bases were easily detected by DGGE in RNA:DNA duplexes of approximately 500 bp. Mobility of the duplexes changed according to the the number, type, and position of the mismatches in the duplexes. We applied this method to detect variations in genomic DNA samples, results being described precisely in the next report, and confirmed that mobility of the duplexes with an identical sequence made from cloned and genomic DNA segments was identical.

B 43

DETECTION OF MISMATCHES IN RNA:DNA DUPLEXES. Report 3. Detection of genetic variants in human genomic DNA by means of denaturing gradient gel electrophoresis(DGGE). Norio Takahashi, Keiko Hiyama, Mieko Kodaira, Hideo Omine, Chiyoko Satoh (Dept. Genet., RERF, Hiroshima)

We have demonstrated that DGGE of RNA:DNA duplexes will detect substitutions, deletions and insertions of bases in DNA (Report 2). We now report the results of our studies in which this method was applied to the direct detection of a single base substitution at position 666 in IVS2 (IVS2-666) of human ß-globin genes in genomic DNA samples. Human ß-globin genes are classified into 4 frameworks. The base at IVS2-666 is T in two and C in the other two. Since this polymorphic substitution is occurring outside of known restriction sites, frameworks usually are determined without direct examination. DNA samples were obtained from established B lymphocyte cell lines from 60 unrelated individuals. Two types of ^{32}P-labeled RNA probes were produced with transcription vectors having inserted IVS2 and exon 3 DNA segments with either T or C at IVS2-666. The frequency of the alleles with C at IVS2-666 was 0.48 and of the alleles with T was 0.52. We also have evaluated variations in other parts of ß-globin genes in genomic DNA samples by means of the DGGE method and have found it to be very effective in screening for mutations at the DNA level.

B 44

STUDIES ON FACTORS INVOLVED IN ACCURATE INITIATION OF HUMAN ß-GLOBIN GENE TRANSCRIPTION. Tatsuo KAWAGUCHI, Shigetaka KITAJIMA, Yukio YASUKOCHI (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo) and Sherman M. WEISSMAN (Dept. Hum. Genet., Yale Univ., CT)

To understand the mechanism of transcriptional regulations, we purified the transcription factors from HeLa cell nuclear extract. We have showed that in addition to RNA polymerase II , six transcription factors (Frs. A, B, C, D and E and stimulatory factor) were necessary for accurate initiation of transcription. This time Fr. A was extensively purified to be nearly homogenous on SDS-PAGE by five successive chromatographic steps on DEAE-Sepharose, phosphocellulose, sulphopropyl (Sp), gel filtration, and carboxymethyl (CM) columns. One peak of absorbance at 280 nm eluted with approximately 0.25M NaCl on CM-HPLC was corresponded to the peak transcription activity. The final preparation showed one band in molecular weight of 27,000 on SDS-PAGE. The activity was detectable in the region of molecular weight of 53,000 on the HPLC gel filtration, indicating that Fr. A existed as a dimer in the aqueous solution. The oligonucleotides deduced from the amino acid sequences for the tryptic peptides of Fr. A were synthesized, and we picked up clones from cDNA as well as genomic DNA.

B 45

IMPLICATION OF GENE CONVERSION IN GENERATION OF POLYMORPHISMS IN THE LINKED FETAL GLOBIN GENES. Satoshi SHIOKAWA, Supan FUCHAROEN, Goonnapa FUCHAROEN, Shunji TOMATSU and Yasuyuki FUKUMAKI (Research Laboratory for Genetic Information, Kyushu University, Fukuoka)

The linked fetal globin genes (the Gγ- and Aγ-globin genes) were cloned from Japanese individuals with three different haplotypes of the *Hin*dIII polymorphisms within the γ-globin genes. Determination of nucleotide sequences of the segment spanning from IVS2 to the 3' flanking region of each γ-globin gene revealed that nucleotide differences are located at 43 positions and a simple sequence stretch of GT or GC. Almost half of the nucleotide changes could be accounted for by gene conversion between the Gγ- and Aγ-globin genes. We found that gene conversion created the *Sac*I polymorphic site just downstream of the Aγ-globin coding region. Association of the *Sac*I polymorphic site with the *Hin*dIII polymorphic site suggests that the region containing these two sites was derived from that of the linked Gγ-globin gene through a gene conversion event. The nucleotide sequences obtained here are identical to those of the Caucasoid fetal globin genes of the same haplotypes, with the exception of some sequence changes in the hot spots of mutations. These results indicate that the sequence heterogeneity of the γ-globin genes can be classified into three major categories according to *Hin*dIII haplotypes. The possible mechanisms of generation of the heterogeneity of the γ-globin gene sequences were presented.

B 46

ORGANIZATION OF THE HUMAN IMMUNOGLOBULIN HEAVY CHAIN LOCUS.
Fumihiko Matsuda, Takayuki Sato, Kwang Ho Lee, Euy Kyun Shin and Tasuku Honjo
(Dept. Med. Chem. Kyoto Univ.)

It is important to examine the precise number of the VH segments, relative orientation of the different VH genes and the overall organization of the immunoglobulin locus in order to elucidate the molecular events underlying the generation of antibody repertoire. We initiated a project which, through direct cloning, aims to construct a physical map of the entire human VH locus. We isolated cosmid clones which cover about 1500 kb regions from human genomic libraries. Further characterization of these regions allowed us to obtain new findings concerning the physical linkage between the VH, D and JH segments.
1. We construct physical maps of the cosmid clones using six rare-site endonucleases. They were classified into 20 independent islands containing in total 63 different VH segments.
2. The VH-VI segment was identified in the 120 kb region which covers D-JH-C-C locus. This segment is located 20 kb upstream of the D4, 5'-most D segment. As we couldn't find any other VH genes in the region between the VH-VI and D4 segment, we concluded that the VH-VI is the most JH proximal VH segment among all known VH families.
3. The chromosomal walking using the VH-VI probe was carried out. We isolated about 30 kb region upstream of the VH-VI segment. No other VH segments were detected in this region.

B 47

CLOSE LINKAGE OF MEN2A WITH RBP3 LOCUS IN JAPANESE KINDREDS. Masayuki YAMAMOTO, Shin-ichiro TAKAI, Kazuyoshi MOTOMURA, Makoto OKAZAKI, Isamu NISHISHO, Hideo TATEISHI, Takesada MORI (The second Dept. Surg., Osaka Univ. Med. Sch., Osaka), and Tetsuro MIKI (Dept. Med. and Geriat., Osaka Univ. Med. Sch., Osaka)

The gene responsible for multiple endocrine neoplasia type 2A (MEN2A), an autosomal dominant disease characterized by medullary thyroid cancer and pheochromocytoma, has recently been assigned to the pericentromeric region on chromosome 10 in European Caucasian kindreds (Mathew et al. 1987, Simpson et al,1987) by linkage analyses using a DNA marker, interstitial retinol-binding protein 3 (RBP3). We also found the tight linkage between MEN2A and RBP3 loci in Japanese MEN2A kindreds. Lod scores were calculated in 5 informative families out of 13 Japanese MEN2A kindreds. The maximum lod score was 5.00 at the recombination fraction of 0.00. This result suggests that mutation of a certain gene close to RBP3 is responsible for MEN2A and no genetic heterogeneity was found in this disease among different races.

B 48

ALLELE FREQUENCIES OF MYCL AND MYB PROTO-ONCOGENES IN UNRELATED HEALTHY JAPANESE. Masako SAITOH[1], Michiko OKADA[1], Naotoshi KANDA[3], Sayuri AIBA[2], Yoshiko SASAMOTO[2], Kura KUBOTA[1] and Yoshiko NOMURA[1] ([1]Chromosome Lab. and [2]Dept. Gynec., Shiseikai Dai-Ni Hosp., Tokyo; [3]Dept. Anat., Tokyo Women's Med. Coll., Tokyo)

The EcoRI RFLPs of MYCL and MYB loci were studied in 51 placentas obtained from the healthy Japanese lying-in women admitted to our hospital in 1987 and the Menderian inheritance was confirmed in the peripheral blood samples obtained from 6 members of a single family. Probes were prepared from the following plasmids: Vector/probe size/cloning site was pJB327/1.8kb/SmaI-EcoRI for MYCL and pBR322/2.6Kb/EcoRI for MYB. The allele frequencies were A1(10kb):A2(6.6kb)=0.41:0.59 at MYCL locus and A1(2.6kb):A2(1.55+1.05kb)=0.44:0.56 at MYB locus. Difference of the frequencies between Japanese and Caucasians was not significant in both loci (MYCL: $p>.7$, MYB: $p>.5$). The observed genotypic frequency in MYCL was significantly different from Hardy-Weinberg expectations ($\chi^2=4.54$, $p<.05$). Co-dominant segregation was shown for MYCL.

B 49

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF THE HUMAN N-myc GENE AND THE SURROUNDING REGION. Yoshito ASHIMURA, Yuuichi NAKAGAWA, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (National Children's Medical Research Center, Taishido, Setagaya, Tokyo 154)

We found two restriction fragment length polymorphisms (RFLPs) in the N-myc oncogene and used the RFLPs for comparing of the genotypes of neuroblastoma patients with normal individuals and for analyses of amplified alleles (Kurosawa et al. ONCOGENE 2:85-90, 1987 & Yamada et al. Jpn. J. Cancer Res. 79:670-673, 1988). In order to extend these analyses, we studied on the RFLPs more precisely and tried to find other RFLPs in or near the N-myc gene. The surrounding region of the N-myc gene was cloned which covered more than 20 kb in both directions from the N-myc gene. Polymorphisms were screened using the cloned DNA fragments as a probe. Nucleotide sequences of a cloned segment were determined including the PvuII polymorphic site. A variant form of the PvuII polymorphism was detected after analysis of about 200 individuals, in which a 5.6 kb fragment was detected instead of 7.1 and 10.1 kb fragments for the P1 and P2 alleles of the PvuII polymorphism.

B 50

CHROMOSOME ASSIGNMENT OF THE PREVIOUSLY UNMAPPED POLYMORPHIC DNA CLONES. Tetsuro MIKI (Dept. Med.& Geriat., Osaka Univ., Osaka), Kenneth K. KIDD, Frank H. RUDDLE, (Dept. Human. Genet., Yale Univ,. USA), Yusuke NAKAMURA (Howard Hughes Med. Inst., Univ. Utah, USA) Isamu NISHISHO, HideoTATEISHI, Takesada MORI, and Shin-ichiro TAKAI (2nd Dept. Surg., Osaka Univ., Osaka)

We isolated 9 different anonymous DNA clones,OS-1~OS-9, which showed RFLPs in Japanese (Jpn. J. Human Genet.31:249,1986). Here we report the chromosome localization of the OS-3,4,6 using both somatic cell hybrid panels and genetic linkage analyses. In the case of the OS-3, Southern blot analysis of a panel of somatic cell hybrids demonstrated that the probe OS-3 was located on chromosome 10. Therefore linkage anaylses were carried out between OS-3 and DNA markers already known to be on chromosome 10 using Yale families. This probe recognized TaqI (A1=6.2, A2=5.5 & 0.7, A3=4.8kb & 0.7 kb), BanII (B1=5.0, B2=3.6 & 1.4kb) and RsaI (C1=1.7, C2=0.9 & 0.8 kb) RFLPs in Caucasians. The A1 allele was the new allele not found in Japanese. There was a strong linkage diseqilibrium among these three RFLPs. The OS-3 (D10S20) was mapped 5.5 cM distal to D10S4 on the long arm of chromosome 10. The other studies demonstrated that the OS-6 (D1S75) was mapped to SPTA (10 cM) on the long arm of chromosome 1 using Yale families and the OS-4 (D18S5) was mapped 28 cM distal to D18S20 on the long arm of chromosome 18 using Utah families.

B 51

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN THE JAPANESE POPULATION. Shin-ichi YOKOTA, Atsushi AKANE, Yoshito ASHIMURA, Yuuichi NAKAGAWA, 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. In addition to the result which we reported at the 32nd Annual Meeting of Japanese Human Genetics Society last year (Suzuki et al.), a total of 60 loci for such RFLP probes have been analyzed. We found that a considerable fraction of the RFLPs were specific to ethnic groups.

B 52

SEVERAL TECHNICAL PROBLEMS IN DETECTION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS. 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 and now widely used in familial and population analyses for genetic diseases as well as for cancer researches. Generally speaking, it is not so difficult to detect RFLPs. However, we sometimes found in literatures and reports crucial mistakes in interpretation of detected DNA fragments which were presumably caused by inadequate experiments. We discussed on such technical problems with respect to the probes which revealed either oligo- or multi-alleles, and also in regard to complete digestion of genomic DNA.

B 53

MITOCHONDRIAL DNA POLYMORPHISM IN THREE JAPANESE POPULATIONS.
Satoshi HORAI, Kenji HAYASAKA and Ei MATSUNAGA
(Dept.Hum.Genet.,Natl.Inst.Genet.,Mishima)

The mitochondrial DNAs (mtDNA) from 64 individuals in Aomori Pref. were analyzed with 24 restriction enzymes. In the analysis of 15 enzymes that recognize 6 base pair, 13 enzymes showed at least two different digestion patterns, while 2 enzymes exhibited monomorphic patterns. Five enzyme morphs were newly observed in Aomori population. Based on a comparison of the cleavage map among the Aomori samples, 20 types of different combination of morphs were observed , of which 9 types were unique in this population. In the anlysis with 9 enzymes that recognize 4 or 5, 43 differnt types were observed. When we estimated the number of nucleotide substitution (d) for each pair of these types, the mean value of d was 0.0026, which is the same as that previously obtained in the Shizuoka population. Comparison of the frequencies of each types among three Japanese populations (Shizuoka, Aomori and Okinawa) revealed signifcant local differences. In contrast to nuclear DNA polymorphism with the same populations which we had already reported, these differeces may reflect the characteristics of mitochondrial DNA such as maternal inheritance.

B 54

APPLICATION OF NAN II TO ANALYSIS OF THE HUMAN GENOME I. Takayuki NAGAOKA (Dept. Cli. Genet., Kyorin Univ., Tokyo) and Shigetaka KITAJIMA and Yukio YASUKOCHI (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo)

We investigated possible application of the enzyme system to analysis of the human genome which is created by the combinations of methylases and Dpn I or Nan II . In order for Nan II to be purified, Neisseria animalis extract was subject to phosphocellulose, Sp, heparin-Sepharose and heparin-5PW column chromatographies. The enzyme preparation showed one major band in molecular weight of 21,000 and two minor bands in the region of molecular weight of 66,000 on SDS-PAGE. On the gel filtration, the enzyme activity was detectable in molecular weight of 20,000, suggesting that the major band on SDS-PAGE was responsible for the Nan II activity. We constructed a plasmid to which Cla I-Cla I linker was inserted at the two sides. When the plasmid was treated with Taq I methylase (New England Biolabs) and then with Dpn I (New England Biolabs and Boehringer Mannheim) or Nan II , the expected 1.4 Kbp and 2.8 Kbp fragments were detected on 1% agarose gel. Neither Taq I methylase nor Dpn I nor Nan II caused the non-specific degradation of the plasmid. Only Nan II , on the contrary, did not caused the non-specific degradation, but the commercialy available Dpn I enzymes did, when the enzymes were applied to the human genome. The Taq I methylase-Nan II digest showed the almost same pattern as the Not I digest on agarose gel.

B 55

GENETIC POLYMORPHISM OF HUMAN DEOXYRIBONUCLEASE I (DNase I)

Koichiro KISHI, Toshihiro YASUDA, Shuichi AWAZU and Keiko MIZUTA
(Dept. Legal Med., Fukui Med. Sch., Fukui)

Deoxyribonuclease I (DNase I) has been studied extensively and has made history in the proteochemical and enzymological fields: the first enzyme to be recognized as specific for DNA, the first DNase to be crystallized, the first DNase for which a specific protein inhibitor was characterized, and so on. However, genetic aspects of DNase I in human systems have received no attention. Recently, we isolated DNase I from human urine and succeeded in producing an antibody specific for the enzyme. The genetic polymorphism of DNase I was studied in human urine samples by polyacrylamide gel isoelectric focusing electrophoresis, followed by immunoblotting using the anti-DNase antibody. Polymorphism easily recognized using this specific antibody, and we therefore propose to designate this new genetic locus *DNASE1*. Four alleles, *DNASE1*1*, **2*, **3* and **4*, have been identified and an autosomal codominant mode of inheritance has been found in the families studied so far. To our knowledge, this is the first report of genetic polymorphism of human DNase.

B 56

全身性エリテマトーデス（SLE）における遺伝要因の解析。緋田めぐみ，森戸文隆，兼岡秀俊，大田明英，小山孝則，山口雅也（佐賀医科大学内科），GENETIC ANALYSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS. Megumi HIIDA, Humitaka MORITO, Hidetoshi KANEOKA, Akihide OHTA, Takanori KOYAMA and Masaya YAMAGUCHI (Dept. Intern. Med., Saga Med. Sch.,Saga)

代表的な自己免疫疾患であるSLEの発症は，種々の遺伝疫学的研究から多因子遺伝によることがわかっている。現時点で，これらのポリジーン系を形成すると考えられる遺伝子座として，CR1,HLA,Gm と，われわれが見出した遺伝子産物である蛋白 Lp1,Lp17, S1（Clin.Chem.34,700,1988）およびRFLPによって検出されるTCR β 鎖定常域遺伝子があげられる。

これらの各因子を数値化し，さらにこれらに種々のweight をつけて得点を計算した。SLE患者と健康人の得点差のt値が最大となるようなweight づけの組合わせは，CR1とS1が4～5，HLA,Lp1,Gmはそれぞれ1，Lp17は0であり，それぞれ単独に計算された相対危険度にほぼ平行していた。

しかし，最大t値に対応するPは10％程度であり，これらの遺伝要因のみでSLEの発症と非発症をすべて区別することはできず，また家系別にみても説明のつけがたい家系が存在した。なおTCR遺伝子単独の相対危険度は5程度で，CR1やS1とともに今後より詳細な検討を要する。またこれらとは別に，新しい有力な遺伝子座の発掘が必要である。

B 57

DNA ANALYSIS OF HEMOPHILIA A

Nobuhiro SUZUKI (Dept. Pediatr., Sapporo Med. Colledge, Sapporo), Takeshi NAGAO (Dept. Hematol., Kanagawa Children's Med. Center, Yokohama), Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (Dept. Congenital Abnormalities Res., Natl. Children's Med. Res. Center, Tokyo)

Hemophilia A, one of the most common inherited bleeding disorders in man, is caused by defects in coagulation factor VIII. Three intragenic and two linked extragenic RFLPs (restriction fragment length polymorphisms) were shown to be useful for carrier detection of this illness. Last year we reported that about 90% of Japanese females were heterozygous for these RFLPs when combined, and we succeeded in carrier detection in 19 out of 22 families at risk for hemophilia A. To date, defects of the factor VIII gene have been characterized at a moleculer level only in less than 70 patients after combination of all the results reported. In the present study, we analyzed DNA samples of 60 patients with hemophilia A to detect factor VIII gene defects at a molecular level. We identified factor VIII gene defects in seven patients : 3 ; deletions, 2 ; point mutations, 1 ; insertion, 1 ; duplication. To our knowledge, the patient with the gene duplication we reported is the first case in hemophilia A.

B 58

Probability of Paternity in Paternity Testings Using DNA Fingerprinting, Masamitsu HONMA, Ikuo ISHIYAMA (Dept. Forens. Med., Univ. of Tokyo., Tokyo)

For the practical use of DNA fingerprinting in paternity testings, we established general formulae based on Bayes' theorem for calculating the probability of paternity. It is supposed that resolvable minisatellite loci in a DNA fingerprint are not linked to one another and that probability of sharing a band between unrelated individuals is x or $2q-q^2$. q is the mean allelic frequency of minisatellite loci. In trio case, paternal fragments can be identified by comparison of the mother's and child's DNA fingerprint. If the number of paternal fragments is n, the probability of paternity is obtained as $W = 1/(1+x^n)$. On the other hand, in the case that paternal fragments can't be identified because of no information from the mother, the correlation coefficient of number of sharing fragments between a parent and the child is used. According to Hardy-Weinberg equilibrium, it can be calculated to $(1+q-q^2)/(2-q)=x'$. Probability that a proportion of bands found in the child's matches those of the parent is calculated on the basis of a binomial distribution: $P={}_nC_r x'^r(1-x')^{n-r}$ and n, the total number of bands; r, Nos. of matching bands. In this case, the probability of paternity is obtained as $W = 1/[1+(x/x')^r\{(1-x)/(1-x')\}^{n-r}]$.

B 59

ACTIVATION OF COMPLEMENT C4 AND PSORIATIC ARTHRITIS

Masahiko MUTO, Hideto KIMURA (Dept. Clin. Genet., Kyushu Univ, Beppu) and Kazunori URABE, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ, Fukuoka)

Psoriatic arthritis (PA) is a psoriasis associated with erosive polyarthritis and usually a negative serological test for rheumatoid factor. PA is considered a genetic disease. We previously reported that PA showed a strong association with HLA-A2-Bw46-DRw8 haplotype. In order to elucidate genetic involvements for development of PA, we performed a population survey on complement C4, which is mapped within HLA region. Serum levels of C4 in the patients with PA were moderately increased, as compared with those of healthy controls. In addition, serum levels of anaphylatoxin C4a derived from C4 in the PA patients were remarkably elevated, as compared with those of healthy controls. Synthesis of C4 molecules is known to be controlled by C4 genes. Then we performed RFLP analysis of C4 genes by using C4cDNA probe with restriction enzymes of EcoRI, HindIII, and BamHI. However, there was no difference in the level of DNA polymorphisms between the PA patients and the healthy controls. These observations suggest that activation of complement C4 is generated in PA. However, it seems likely that this event is perhaps due to disorders in its regulatory mechanisms.

B 60

Gγ-GLOBIN GENE EXPRESSION IN A JAPANESE WITH ELEVATED FETAL HEMOGLOBIN LEVEL IS ASSOCIATED WITH A SPECIFIC SUBHAPLOTYPE 5' TO THE δ-GLOBIN GENE.

Koji SHIMIZU and Hiromi KEINO (Dept. Morph., Inst. Develop. Res., Aichi Prefect. Colony, Kasugai, Aichi)

The Gγ values in fetal hemoglobin (Hb F) of healthy Japanese blood donors with elevated Hb F level (0.8-11%) were analysed. They did not show any detectable large deletions in the β-globin gene cluster, nor abnormal γ-globin gene arrangements, by restriction endonuclease mapping. Individuals homozygous for a subhaplotype [+-----] 5' to the δ-globin gene (Hinc II 5' to ε; Xmn I 5' to Gγ; Hind III in Gγ; Hind III in Aγ; Hinc II in ψβ1; and Hinc II 3' to ψβ1) had low to mid Gγ values (7-49%), while those for [-++-++] possessed high Gγ values (58-85%). Those heterozygous for [+] and [-] at the Xmn I site 5' to (-158 base pairs of the cap site of) the Gγ-globin gene showed mid to high Gγ values (45-65%). Three heterozygotes for [+-----] and [-++-++] had 45, 46, and 53 %Gγ. In conclusion, a subhaplotype [-++-++] may be highly associated with the determinant for the high Gγ-globin gene expression, while a subhaplotype [+-----] may not be highly associated with that for the low Gγ-globin gene expression. Increased expression of the Gγ- or Aγ-globin gene can occur without marked increase of Hb F level.

B 61

AMINO ACID SUBSTITUTIONS DETERMINING G3m(g5).

Shigenori ITO, Kiyoshi FUJITA, Tokiko MIYAZAKI, Hideo MATSUMOTO (Dept. Legal Med., Osaka Med. Coll., Takatsuki)

Allotypic G3m(g5) marker specific substitutions were studied by sequence analysis of C-terminal BrCN peptides derived from two myeloma proteins, Ba(Gm(g,g5)) and Bu (Gm(b0,b1,b3). The experimental results indicate that two residues are responsible for the specificity. IgG3 protein carrying G3m(g5) has arginine at position 435 and tyrosine at position 436, whereas protein carrying G3m(b0) has Arg-Phe in these positions. IgG3 carrying G3m(s), which is tightly bound to protein A, has His-Tyr. This study shows that the amino acids at position 435 and 436 play an important role on epitopes of Gm marker and protein A interaction.

B 62

REACTIVITY OF E BLOOD GROUP ANTIGEN WITH ANTI-H AGGLUTININS AND RELATED OLIGOSACCHARIDE STRUCTURES OF THE ANTIGEN.

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

E blood groups found by Sugishita in 1935 were classified group A, B and AB red cells into high reactive E and low reactive e groups with eel type II anti-H serum. Reactivity of E and e red cells was tested with anti-H agglutinins of various sera and lecins. Eel type I serum which agglutinin was inhibited by L-fucose as same as type II eel serum showed the same reactivity with E and e red cells. Though the agglutination of Ulex I was inhibited by L-fucose and that of Ulex II by N-acetylchitobiose, both lectins differentiated E and e red cells. Human anti-H antibody found in human group Ae serum which was difficult to inhibit the reaction with group O red cells by L-fucose strongly agglutinated E red cells. The agglutination of group O red cell immune chicken serum was not inhibited by L-fucose and the anti-H showed same reactivity with E and e red cells.

As all group O and subgroup A_2 belong to E the oligosaccharide sequence of H active structures reported by Hakomori suggest the possibility that the type 3 chain H would be quantitative difference between E and e in group A red cells.

B 63

DISTRIBUTION OF HUMAN IMMUNOGLOBULIN G HAPLOTYPES AMONG SOME EUROPEAN POPULATIONS.

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The genetic markers of IgG (Gm allotypes) are inherited in fixed combinations termed Gm haplotypes. So far, it has been recognized that the Gm haplotypes common to Mongoloid populations are Gm ag, axg, ab3st, and afb1b3; among Caucasoids, Gm ag, axg, and fb1b3; and among Negroids, Gm ab1b3, ab1c, and ab3s. Either four or five Gm haplotypes characteristic of Caucasoids and Mongoloids were observed among the three Hungarian populations (Szekely, Paloc, and Jews), on the other hand, the Hungarian Gypsy has five Gm haplotypes as well, although in extremely high frequency of Gm afb1b3 (21%) characteristic of southern Mongoloids and rather high incidence of Gm ab3st (5.5%) in comparison to any other European populations. The Gm data of Hungarian Gypsy shows that they are quite unique ethnic group among European populations, suggesting that the origin of Gypsy must exists in northern India. Spanishes from Catalonia have four Gm haplotypes including Gm afb1b3 (3%) characteristic of southern Mongoloids, suggesting some admixture with southern Mongoloids.

B 64

THE SECOND CASE OF REVERSION MUTATION FROM HP* 2 TO HP* 1 FOUND IN A JAPANESE.

Jun-ichi ASAKAWA, Mieko KODAIRA, Nori NAKAMURA, Eiko NISHIKORI, Chiyoko SATOH, (Dept. Genet., RERF, Hiroshima)

Among a total of 23,326 Japanese examined for plasma haptoglobin (HP) by electrophoresis, one HP Carlberg variant whose pattern resembles that of a mixture of HP 1-2 and HP 2 was found in a woman. Her plasma HP level was normal, although the molar ratio of HP α1 to HP α2 polypeptide was 1/3. Her mother showed HP 2 phenotype and the father was HP 1-2, and both of their plasma HP levels were normal. These observations suggest that she is mosaic for HP 2 and HP 1-2 cells. Her peripheral B cells were transformed by EB virus and cloned in agarose. Southern blot analysis of the DNA from each colony revealed that 9 among 20 colonies showed HP* 2/HP* 2 and 11 showed HP* 1/HP* 2 genotype. The HP* 2 gene is formed by a partial duplication within two HP* 1 genes. The results suggested that originally the propositus received two HP* 2 genes from the parents but a reverse mutation from HP* 2 to HP* 1 occurred one of the HP* 2 genes at an early stage of embryogenesis, probably the two-cell stage. The mutation seemed most probably resulted from intra-locus homologous recombination and consequent deletion of half of the duplicated segment of HP* 2 gene. This is the second case of the reverse mutation from HP* 2 to HP* 1 detected in our study.

B 65

Dentato rubro pallido luysian atrophy (DRPLA) の一家系 —— 臨床像の多様性の検討. 今井薫・山岡光子・三石洋一・泉達郎・福山幸夫（東女医大・小児科）.
DRPLA: A FAMILY STUDY AND ITS CLINICAL HETEROGENEITY. Kaoru IMAI, Mitsuko YAMAOKA, Yoichi MITSUISHI, Tatsuro IZUMI and Yukio FUKUYAMA (Department of Pediatrics, Tokyo Women's Medical College, Tokyo, Japan)

精神遅滞(M.R.)失調歩行，痙攣．choreoathetosis を呈した小児例（７歳，男Ⅴ－１）を発端者として，５世代９症例からなる常染色体性優性遺伝を示すと思われたDRPLA の一家系を報告した。第一世代は同一地域の出身，Ⅴ－１の父方祖母（Ⅲ－１）は失調歩行のみを認め，Ⅲ－２は４歳より失調歩行，痴呆choreoathetosis を認め，他院でDRPLA と診断，Ⅲ－５は３８歳より失調歩行，痙攣，性格変化を認め，他院でDRPLA と診断されている。Ⅲ－６は，失調歩行，痙攣，痴呆を認め，４４歳で死亡。Ⅳ－４は核黄疸で死亡。Ⅳ－５とⅣ－６は精神遅滞を認め幼児期死亡。Ⅳ－10は痴呆，失調歩行，痙攣を認める。本症の父（Ⅳ－２）は軽度小脳失調のみで，多幸性である。臨床像，経過に多様性がみられ，早期発症例は精神遅滞，成人期発症例は歩行障害が主症状をなし，性格変化は初期には多幸性を示し，進行とともに粗暴傾向を示した。先行症状としては早期発症例は，知能障害，遅発例では歩行障害であり，遅発例では歩行障害の前に多幸性を示しており，性格変化にも注目すべきである。

B 66

２遺伝子座模型の分離比分析．安田徳一・伊藤綽子(放医研・遺伝)．COMPLEX SEGREGATION ANALYSIS FOR TWO-LOCUS-MODELS. Norikazu Yasuda and Hiroko Ito (Division of Genetics, National Institute of Radiological Sciences, Chiba)

ヒトが70歳までに罹病する遺伝素因のある疾患は新生児コーホトの６割に達するといわれるが、これらの多くの遺伝様式は不明、もしくは多因子性であると考えられている。これら難病に主効果を表わす遺伝子を検索して、遺伝様式を確立し遺伝相談や病因解析の手掛りを得る目的で、２遺伝子座による分離比分析の模型とその電算機プログラムの開発を試みた。資料は患者を発端として健常者も調べた両親と子の核家族である。各座位に正常、異常の２対立遺伝子を仮定すると、健常者、患者の２表現型と２座位による遺伝子型の対応は理論的に50通りある。今回は２重劣性模型、相補優性模型、エピスタシス模型、優性修飾模型、ポリジーン模型、”利き手”模型の６模型を開発した。２遺伝子座模型は１遺伝子座模型に比べて両親の表現型からの子の分離比が２種類以上になる。これらの複合分離比は各座位の疾患遺伝子頻度で表される関数（たとえば２重劣性模型では２疾患遺伝子頻度の調和平均）で加重する形になるので、それらをなんらかの方法で求める必要がある。確認法の補正後、フェニルケトン尿症、先天性副腎過形成、小頭症、聾、白子等については単純劣性遺伝で十分説明でき、乾せんについては異質性の他に２重劣性遺伝が示唆され、２遺伝子座の関与が考えられる。スギ花粉症は浸透率が約70%で単劣性仮説にほぼ合うが、２重劣性の模型も可能性として残った。

B 67

ISOLATION OF cDNAS ENCODING THE M AND B ISOFORM OF HUMAN PHOSPHO-GLYCERATE MUTASE (PGAM) AND CHARACTERIZATION OF THE PGAM GENE FAMILY.
Saburo SAKODA, Seiichi TSUZINO, Ryuzo MIZUNO, Tsutomu AZUMA, Susumu KISHIMOTO (3rd Dept. Intern. Med., Osaka Univ. Hospital, Osaka), Tomokazu SUZUKI (Dept. Clin. Genet., Med. Inst. Bioregulation, Kyushu Univ., Beppu), Sara SHANSKE, Eric A. SCHON, Salvatore DiMAURO (Neurol. Inst., Columbia Univ., N.Y.C.)

The isolation of cDNAs specifying the muscle- and brain-specific isoforms of phosphoglycerate mutase (PGAM-M and -B respectively) revealed that PGAM-B encodes a deduced protein 254 amino acid long, 79% identical to PGAM-M and contains a 913-nucleotide (nt) 3' untranslated region, as compared to the unusually short 37-nt 3' untranslated region of PGAM-M. Nothern analysis demonstrates the muscle-specific nature of PGAM-M and the non-muscle-specific nature of PGAM-B. Genomic Southern analysis implies the presence of a large PGAM family in the human genome. Most of PGAM-hybridizing sequences seem to the related to the B isozyme gene, probably the processed pseudogenes. These result agree with the evolutionary analysis, which indicates that the PGAM-B gene is the progenitor of the M gene.

B 68

MAN'S PLACE BASED ON THE GENE.
Shintaroh UEDA, Yoshihisa WATANABE, Naruya SAITOU, Keiichi OMOTO (Tokyo Univ., Tokyo), Hidenori HAYASHIDA, Takashi MIYATA (Kyusyu Univ., Fukuoka), Hiroshi HISAJIMA, Fumihiko MATSUDA and Tasuku HONJO (Kyoto Univ., Kyoto)

To know man's place (phylogenetic relationships among hominoids), immunoglobulin epsilon and alpha genes of chimpanzees, gorillas and orangutans were isolated and their structures were compared with the human counterparts. (1) Multiple deletions and duplications seem to have happened in both genes during hominoid evolution; chimpanzees had deleted the entire epsilon 2 gene after its divergence. In addition, the length of the alpha 1 hinge region of gorillas is distinct from those of chimpanzees and humans. Structural homology of the epsilon and alpha genes suggests that humans are evolutionarily closer to chimpanzees than to gorillas. (2) The comparison of the nucleotide sequences of epsilon 3 processed pseudogenes chose chimpanzees at the most close relative to humans, although the degrees of nucleotide differences had no statistical significance among humans and the African apes. This result was consistent with that deduced by the above qualitative study.

C 1

CARRIER DIAGNOSIS OF NORRIE DISEASE USING DNA PROBE L1.28(FIRST DEMONSTRAION OF RECOMBINATION)Susumu KATAYAMA,Akira USUI, Harumi KUBO(Dept. Obstet.Gynecol.,Toho Univ.,Tokyo and Mitchell S. GOLBUS(Repro.Genet., Univ. California,San Francisco.,USA)

Norrie disease (ND) is a rare X-linked recessive disorder. About 300 patients have been reported throughout the world, 4 patients in Japan. ND is thought to be tightly linked to anonymous probe L1.28. We report the first recombination event among 24 informative meiosis. DNA,extracted from peripheral white blood cells was digested with TaqI endonuclease and electrophresed followed by transfer from the gel to nitrocellulose filter using modified Southern protocol. The filter was hybridized to nick-translated L1.28 probe. The family has 2 affected males. One affected male had the 9 kb allele which was one of two 9kb alleles found in his mother.This 9 kb was associated with the X chromosome which carried the ND gene.His sisiter had 2 different alleles,a 12 kb allele from her father and 9 kb allele from her mother which was shared with her affected brother. However her affected son did not have this 9 kb allele but rather had 12 kb allele instead. This can be explained only by meiotic recombination between the mother's two X chromosomes . This indicate there is a 4%(95%C.L. 0.1-21%) error rate introduced by meiotic crossover in carrier or prenatal diagnosis based on linkage between ND and L1.28.

C 2

表皮線維芽細胞に相互転座が認められた大腸癌症例．山田秀人[1,2]・森田裕之[1]・児井穏[1]・押村光雄[1]（[1]神奈川がんセ・研・細胞遺伝，[2]北大・医・産婦）．CHROMOSOME REARRANGEMENTS IN CULTURED SKIN FIBROBLASTS FROM PATIENTS WITH COLON CARCINOMA. Hideto YAMADA[1,2], Hiroyuki MORITA[1], Minoru KOI[1] and Mitsuo OSHIMURA[1] ([1]Lab. Cytogenet. Kanagawa Cancer Center Res.Inst.,Yokohama; [2]Dept. Obstet. Gynecol., Hokkaido Univ., Sapporo)

大腸癌好発疾患の家族性大腸ポリポーシス患者由来の表皮線維芽細胞に染色体異常が高頻度に観察されることが報告されており，発癌に至り易い遺伝的素因との関連が示唆されている．今回我々は，家族歴をもたない大腸癌５症例における表皮線維芽細胞の染色体分析の結果，１症例で高頻度に染色体構造異常が認められたので報告する．

症例は57歳，男性．StageⅤ，DukesCのS状結腸癌で肝多発性転移，リンパ節転移が認められた．肘部から表皮線維芽細胞を培養し，培養２－３代目に染色体分析を施行した．右肘部からの培養細胞：148分裂中期像のうち49細胞(約33%)で染色体構造異常が認められ，t(1;13)(p1;p1)が 34細胞に，t(1;11)(p2;p1)が 14細胞に，t(1;13)(p3;q1)が１細胞に認められた．また，左肘部からの培養細胞：82 分裂中期像のうち７細胞(約９%) で染色体構造異常が認められ，inv(2)(p1q2) が３細胞に，t(1q-;10p+;13q+) が２細胞に，t(8;X)(p2;q2)が１細胞に，t(8;X)(q1;p2)が１細胞に認められた．

以上のことより，非家族性の大腸癌症例においても発癌に至りやすい遺伝的素因と染色体不安定性の増大との関連が示唆される．

C 3

ISOELECTRIC FOCUSING ANALYSIS OF HUMAN PLASMA ZN-α2-GLYCOPROTEIN VARIANTS. Nori NAKAYASHIKI and Syusaku KATSURA (Dept. Legal Med., Sch. Med., Iwate Med. Univ., Iwate)

Zn-α2-glycoprotein (ZAG) was first isolated from human plasma by Bürgi and Schmid (1961), and its complete amino acid sequence was determined by Araki et al.(1988). Kamboh and Ferrell (1986) examined ZAG of sera or plasma from three races in the U.S. population (N=445) by means of a polyacrylamide gel isoelectric focusing followed by immunoblotting. A variant of ZAG was found in one Black individual, but the genetic background could not be studied.

In this report, desialyzed plasma samples from 610 healthy adults in Japan were tested. A single ZAG band appeared in 601 samples (named as ZAG 1), while another 9 samples consisted of double bands containing the ZAG 1 band. These double bands were classified into three types (ZAG 2-1, ZAG 3-1 and ZAG 4-1). The calculated allele frequencies of ZAG*1, ZAG*2, ZAG*3 and ZAG*4 were 0.9926, 0.0033, 0.0033 and 0.0008, respectively. Family studies of two rare types, ZAG 3-1 and ZAG 4-1, indicated the existence of its genetic transmission. Moreover, ZAG patterns of various human body fluids (urine, sweat, saliva, seminal fluid and vaginal secretion) were tested by IEF or SDS-PAGE and compared to the plasma ZAG.

C 5

FRAGILE SITES IN NEWBORNS AND AGED WOMEN. Yoko WATANABE, Tetsuji KADOTANI and Noriko KUROSAKI(The Kadotani Med. Res. Found., Hiroshima)

Chromosome fragile sites were studied on 29 cases of newborns and 30 cases of aged women who were clinically normal. The lymphocytes were cultured for 72 hrs in the medium MEM without folic acid supplemented with 5 % calf serum. A part of the cells were exposed to caffeine. The slides were made by means of the flame-drying technique. The chromosome observation was made the frequency of the break and/or gap with 50 well-delineated metaphases for each by the culture with and without caffeine. The locations of the break points were identified by conventional Giemsa and G-banding staining after destain.

The fragile sites were observed with a little increase in the medium with caffeine. The frequencies of the fragile sites in the aged women were a little higher than newborn group in the medium with caffeine, but it's not significant. In the group of aged women, much more types of the fragile site were observed. It was interesting finding that the X-chromosome in the medium MEM without folic acid with caffeine revealed a fragile site on p22 in clinically normal aged women. The incidence of break points was different in the individual case.

C 6

A CASE OF FAMILIAL FRAGILE SITE 12q24. Kazumi IKAWA, Emiko NAKAYAMA, Misako WATANABE, Hiroko MATHUZAKI, Miki YAZIMA (Ishikawa Health Service, Ishikawa), Naoto UWADANA (Kaga-city Hosp.,Ishikawa), Hiroko kawashima (Dept. Pediatr., Kanazawa Univ., Ishikawa) and Shigeru MARUYAMA (Kanazawa Holy Spirit Hosp., Ishikawa)

We present a case of familial fragile site. Eight-year-old male was reffered to us because of his mental retardation. He has depressive father and shizophrenic maternal uncle. His clinical findings include speech delay, mild autistic behavior and large stature (Ht +3.3SD, Wt +4.0SD, Hc +1.0SD).

Although, routine chromosome test were normal in patient and his parents, fragile site 12q24 was observed in 3~11% of mitoses from the culture with MTX in both patient and his father.

A sporadic case of fragile site 12q24 was reported in a mentally retardede male, however, no familial case has been reported except ours.

C 7

KARYOTYPIC ANALYSES IN DESMOID TUMORS DEVELOPED IN PATIENTS WITH FAMILIAL POLYPOSIS COLI.

Mitsuaki A. YOSHIDA, Tatsuro IKEUCHI, Akira TONOMURA(Dept. Cytogenet., Med. Res. Inst., Tokyo Med.& Dent. Univ., Tokyo), Michiko MIYAKI, Takeo MORI(Tokyo Metro. Inst. of Med. Sci., Tokyo), Takeo IWAMA(Kyoundo Hosp. Tokyo), Yasuhide USHIJIMA and Akio HARA (National Saitama Hosp.)

Chromosome analysis was successfully performed on desmoid tumors obtained from 2 female patients with familial polyposis coli (FPC). Both tumors showed a modal chromosome number of 46. Ten metaphases were karyotyped in Case 1, and del(5)(q21q31) was identified in 9 cells. In 3 cells of them, the del(5) was a sole abnormality, and some additional changes in structure and number were observed in the other 6 cells. Although all of metaphases analysed showed a 15p+, this chromosome was observed in both peripheral lymphocytes and skin fibroblasts from the patient, indicating that this was a heteromorphic chromosome. In Case 2, 24 out of 28 metaphases analysed showed a normal karyotype, and rearrangements involving 17q were identified in 2 of the remaining 4 cells. Recently, the FPC gene was localized to 5q21-22 by linkage analysis in FPC families, and a loss of heterozygosity in this region was frequently observed in FPC tumors as well as in sporadic colon tumors. The fact that an interstitial deletion of 5q was detected in one of two desmoid tumorsis of particular interest for better understanding the mechanism of tumorigenesis in FPC

C 8

GENE MAPPING OF FOUR DNA SEGMENTS NEAR THE WAGR COMPLEX LOCUS BY in situ HYBRIDIZATION. Yoshimitsu FUKUSHIMA (Div. Med. Genet., Saitama Child. Med. Ctr., Saitama), Lisa M. DAVIS and Thomas B. SHOWS (Dept. Human Genet., Roswell Park Memorial Institute, New York)

The majority of patients with the Wilms' tumor-aniridia-genitourinary-mental retardation (WAGR) complex have hemizygous constitutional deletion of 11p13. The gene of the WAGR complex has assigned to 11p13. 112 human single copy DNA clones were isolated from chromosome 11 library and mapped to chromosome 11 by Davis et al. (Genomics, 1988). 12 of these clones mapped around 11p13. We report the precise localization of four of these 12 clones (530, 589, 706, 1027), using in situ hybridization.

The DNA segment ^{3}H-labeled by random priming method was hybridized to normal human prometaphase lymphocyte chromosomes. The labeled probe produced silver grains after autoradiography for 5-7 days at the site of chromosomal homology, and chromosomes were then stained for G-banding. The scanning of 100 metaphase spreads showed that the majority of grains were localized around 11p13 in all four DNA clones. The peak of grains was 11p14.1 in 1027, the distal region of 11p13 in 706 and 530, and the proximal region of 11p13 in 589. These results were compatible with those of Davis et al. (Science 241:840-842, 1988).

C 9

ANALYSIS OF BREAKPOINT CLUSTER REGION (BCR) IN CHRONIC MYELOGENOUS LEUKEMIA (CML) WITH VARIANT PHILADELPHIA (Ph^{1}) TRANSLOCATION. Hikari NISHIGAKI, Tsukasa OKUDA, Shigeo HORIIKE, Shoichiro TSUDA, Masafumi TANIWAKI, Shinichi MISAWA, Tatsuro TAKINO (3rd Dept. Int. Med., Kyoto Pref. Univ. Med., Kyoto), Johji INAZAWA and Tatsuo ABE (Dept. Hygiene, Kyoto Pref. Univ. Med., Kyoto)

We examined the bcr-rearrangement in six patients with variant Ph^{1} and 21 with standard Ph^{1}. Southern blot hybridization using 5' and 3' bcr probe detected a bcr-rearrangement in five of six patients with variant Ph^{1} and in all of the patients with standard Ph^{1}.

Five of six patients with variant Ph^{1} had a breakpoint at 5' region in bcr (upstream to the second HindIII site). Eleven of 21 patients (52.4%) with standard Ph^{1} had a breakpoint at 5' region in bcr and the remaining 10 patients (47.6%) at 3' region.

The deletion within bcr sequence was found in three patients (60%) of variant Ph^{1} and in three (14.3%) of standard Ph^{1}. Thus, there may be a significant difference in patients with variant Ph^{1} from those with standard Ph^{1} with respect to the pattern of bcr-rearrangement.

C 10

CHROMOSOME FEATURES OF 301 CASES OF MALIGNANT HEMATOLOGICAL DISORDERS STUDIED IN OUR LABORATORY FOR THE PAST 3 YEARS. Masako MINAMIHISAMATSU and Takaaki ISHIHARA (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba)

The results of the chromosome analyses in 301 cases of leukemias and related hematological disorders for a limited period of three years from 1985 through 1987 are reported. Chromosome abnormalities were observed in 159 (52.8%) of the 301 cases. The distribution of all the breakpoints of the structural chromosome abnormalities except those of specific translocations such as t(9;22) in CML, t(8;21) in AML and t(15;17) in APL and that of numerical chromosome aberrations to the 22 pairs of autosomes and the sex chromosomes was investigated in order to find out about the significance of aberrations other than the well-known chromosome abnormalities. The breakpoints of 201 breaks observed were distributed to 91 bands, especially frequently to 2q33, 5q13, 7q22, 9q22, 11q23, 12p13, 14q32, 17p11 and 20q11. With regard to numerical chromosome aberrations, the increase in #8 chromosomes and the decrease in #7, #17 and Y chromosomes were observed in high frequencies. The results of the present chromosome analyses suggest that certain specific chromosomes or their specific loci may play a significant role in the genesis and/or progression of human malignant blood disorders.

C 11

CASE OF 4q3 TRISOMY. Masafumi HANDA, Noriko MATSUMOTO, Hiromi SAKAMOTO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med., Nishinomiya), Kiyoko NISHIMURA, Hiroko TSUKAMOTO, Megumi MIYAKE (Dept. Pediat., Palmore Hosp., Kobe) Yoshie SUGAHARA and Hiroko MIMURA (Dept. Clin. Genet., Hyogo Coll. Med., Nishinomiya)

The proband, male infant, was born on March 19, 1988 as first living child of 28-year-old father and 27-year-old mother. Their first pregnancy aborted spontaneously at 10 weeks' gestation. this pregnancy was complicated by threatened abortion at 3 months' gestation. He was born at 39 weeks' gestation. His birth weight was 3000g and cyanotic and edematous over the entire body. At the age of one month, he weighted 3700g and was 49cm long. Physical examination revealed bilateral chest depression at inspiration and following abnormalities; quadranguler face, small chin, epicanthic folds, hypertelorism, relatively prominent nose, low set ear, high arched plate, abundant and loose skin at the neck, club feet and rocker-bottom feet. At the age of 5 months, he weighted 6200g and was 59cm in length. He was hypotonic and could hold his head for a short time. Cardiac malformation and kidney deformities were not found. His father had a normal karyotype. His mother had a karyotype of 46,XX,t(4;18)(q31.3;q23). His karyotype was revealed 46,XY,del(18),t(4;18)(q31.3;q23)mat and trisomy from 4q31.3 to qter.

C 12

INVERSION OF CHROMOSOME 4(p14p16.1) IN THE FATHER OF CHILD WITH REGULAR TRISOMY 21. Mashio KITATANI, Mamoru OZAKI, Etsuko TAKASE, Hiroaki TAKAHASHI (dept. Clin. Genet., Inst Hum. Genet., Kanazawa Med. Univ., Ishikawa)

A case of a paracentric inversion of chromosome 4 was found in the healthy father of child with regular trisomy 21. The karyotype is 46, XY, inv(4)(p14p16.1). The affected child was the second child born to a young couple. The first daughter with cleft palate carried the same inversion as her fathers. The parental origin of the extra chromosome 21 could not be determined. To date, inversion chromosome 4(p14p16.1) has been reported rather infrequently. There have been only case of inversion chromosome 4 when ascertained by advanced maternal-age prenatal genetic study. There was no particular phenotype which identified this inversion. Therefor it become necessary for us to further asses the implication for genetic counselling for carriers of inversion chromosome 4(p14p16.1).

C 13

遺伝子量効果を応用したde novo 12pトリソミーの診断. 笠井良造[1]・楢原幸二[2]・松原恒則[3]（[1]旭川児童院、[2]岡大・医・小児、[3]岡大・医・癌研生化学）.
DIAGNOSIS OF THE CASE WITH A DE NOVO 12p TRISOMY USING GENE DOSAGE EFFECTS FOR LDH AND GAPD. Ryozo KASAI[1], Kouji NARAHARA[2] and Tsunenori MATSUBARA[3] ([1]Asahigawa Jidoin Hosp., Okayama, and [2]Dept. Pediatr., and [3]Cancer Res. Unit, Okayama Univ., Okayama).

遺伝子量効果の研究を応用して診断したde novo 12ｐトリソミーの１例を報告した。症例は２歳１カ月の男児。理学所見として、大頭症、前頭部突出、後頭部扁平、小さく変形した耳介、耳介低位、広く低い鼻根、短い鼻、大きな頬、魚様の口、下口唇外反、高口蓋、歯列不整、副乳、臍ヘルニア、右停留睾丸、短い指など、12ｐトリソミー症候群に特徴的な臨床症状が認められた。ＤＱは42で重度の精神運動発達遅延を示した。染色体検査で両親の核型は正常であったが症例は 46,XY,11q+ を呈し、過剰分節は 12pter→p11.2 と推測された。症例および両親の赤血球を用いて、12p12および13に遺伝子座位が決定されている LDHおよびGAPD の活性を Beutler 法により測定したところ、症例はいずれも正常に比し1.5倍の遺伝子量効果が認められた。以上の結果より、症例の核型は 46,XX,-11,+der(11),t(11;12)(q25;p11.21)de novo と考えられた。

de novo の染色体構造異常の診断には、分染法による同定のみならず、該当する染色体分節に存在する遺伝標識あるいはＤＮＡの遺伝子量効果による確認が必要であろう。

C 14

Tow Siblings with Partial 13pter→q13 Trisomy and 21pter→q21 Monosomy, and Congenital Multiple Arthrogryposis in Parents with Deafness.

Naoki NOMOTO, Yuri MIYANOMAE, Kazuhisa ISHIMURA (Dept. Pediat., Kyoto City Child Welfare Center, kyoto), Osamu NAGAUCHI (Dept. Clin. Labo., kyoto City Hosp., Kyoto), Yuriko YAMORI (Dept. Neuropediat., St. Joseph's Hosp. for Handicapped, kyoto) and Hitoshi OKAZAKI (Dept. Pediat., Kansai Med. Coll., Osaka)

Tow siblings were the product of full term pregnancy born to a woman(gravidaⅢ, paraⅡ). Elder sister was 2260g at birth when her mother was 32 year old and father 35 year old, and brother was 2750g and his mother was 35 year old and father was 38 year old at birth. Their previous clinical diagnosis was congenital multiple arthrogryposis. Their common features included low birth weight, poor feeding, hypotonicity, sever developmental and mental retardation, narrow temple, large almond like eyes, antimogoloid slant, hypertelolism, round and broad nasal tip, high arched palate, cleft palate, large low set ears, slant tip of all fingers, bilateral simian creases, hypoplastic dermoglyphic ridge, and contraction of joints. On one hand, elder sister had mild deafness and brother had heavy inguinal hernia, cryptorchism and ventricular septal defect.

Using G-, Q-, R-banding methods, the mother's karyotype was 46,XX,t(13;21)(q13;q21), whilet hat of tow siblings were 46,XXorXY,-21,+der(21)t(13;21)(q13;q21)mat. The father's karyotype was normal. It was made certain by normal SOD-1 value (by Cytochrome C method and monoclonal antibody method) of the parents and tow siblings that the mother's karyotype was 46,XX,t(13;21)(q13;q21).

C 15

A CASE OF AN INTERSTITIAL DELETION OF 15q 46,XY,del(15)(q15q21.2)

Keiko WAKUI, Toshiro NISHIDA (Dept. Clin. Lab., Saitama Child. Med. Ctr., Saitama), Hiroshi NISHIMOTO (Div. Neurosurg.) and Yoshimitsu FUKUSHIMA (Div. Med. Genet.)

The patient was a 1y8m-old boy. His mother was 35y and father 34y at his birth. They were healthy and not consanguinous. The mother noticed polyhydramnios and deminished fetal movement during the pregnancy. He was born at 39 weeks' gestation. His birth weight was 2,070 g. His clinical features were the followings: poor weight gain, developmental delay (DQ 22), craniosynostosis (metopic and sagittal synostosis), turricephaly, arched eyeblows, shallow orbit, flat nasal root, retrognathia, high arched palate, large ears, funnel chest, cryptorchidism, tappering fingers, bilateral simian crease and prominent heels. High-resolution chromosome analyses revealed that his karyotype was 46,XY,del(15)(q15q21.2). The parents had a normal karyotype. The study of heteromorphic markers of chromosome 15 showed the deleted chromosome 15 was paternal origin.

Only two cases with the monosomy of the middle portion of 15q have been reported. They did not suffered from craniosynostosis and their deleted region did not include 15q15. Thus, the chromosome band 15q15 might be an important segment for craniosynostosis.

C 16

CLINICAL AND CYTOGENETIC STUDIES OF TETRASOMY 18P.

Tomoko HASEGAWA, Koichi ENDO, Tsunehiro YOKOCHI (Shizuoka Childrens' Hospital, Shizuoka), Hitoshi NAKASHIMA and Takashi IMAMURA (National Institute of Genetics, Mishima)

We report on the five (two males and three females) cases who were clinically and cytogenetically diagnosed as tetrasomy 18p. All of the patients showed psychomotor retardation, dolichocephaly, delicate but only slightly anomalous facial appearance, low set and small ears. Limited joint movements were observed in four patients. Chromosomal studies by the G-banding method revealed one tiny extra-metacentric chromosome in all five cases. Both arms of each extra-metacentric chromosome were very similar to the short arm of chromosome 18. By means of methods described by Lawrence et al. (1988), biotin-labeled probe L1.84 (Devilee, 1986) specific for the pericentric region of chromosome 18 was hybridized in situ to metaphase chromosomes and interphase nuclei of the EB-transformed lymphoblasts established from these patients. Hybridization resulted in three bright fluorescein spots in each metaphase figure and nucleus. All exhibited labeling on the centromere of minute extra-chromosome, and in all cases both chromosomes 18 were labeled. We tentatively concluded that the minute extra-metacentric chromosome was i(18p). One female patient without joint limitations had mosaicism of 46,XX/47,XX,+i(18p).

C 17

PRENATAL DIAGNOSES OF CHROMOSOMAL ABNORMALITIES:

Hisashi HAGIWARA(School of Health Science, Kyorin Univ., Tokyo), Michiko OHNO, Tomoko FUNATO, Akira MATSUNOBU, Kyoko YOSHIHARA, Noriko YABE, Tohru MAEDA(Dept. Lab. Med., Kitasato Univ., Kanagawa)

The results of amniocentesis for the intrauterine diagnosis of chromosome abnormalities were presented in detail. These observations were collected from 1610 consecutive prenatal diagnoses monitored in our laboratory from 1973 to 1988. Chromosome preparations were made by "in situ" method on the cells grown on the coverslips. Autosomal aneuploidy was found in 16 fetuses(1.0%) including 12 with trisomy 21, 2 with trisomy 18, 1 with trisomy 13 and 1 with a supernumerary small marker chromosome. Sex chromosome abnormalities were found in 2 fetuses, 1 with 47,XYY and 1 with 46,X,i(Xq) karyotype. There were 20 balanced chromosomal rearrangements(1.2%) including 10 reciprocal translocations, 9 robertsonian translocations and 1 pericentric inversion of chromosome 7. Twenty-four prengancies(1.49%) ended in spontaneous abortion within 4 weeks following amnicentesis. The number of amniocenteses performed annually has been increasing exponentially in recent years, the greatest increase being in the advanced maternal-age group.

C 18

FAMILIAL CHROMOSOMAL TRANSLOCATION t(1,15) AND PERICENTRIC INVERSION 9. Hideki KURIKI, Kenjiro MURATA (Dept. Clinico-labo., Kansai Med. Univ., Osaka), Sadako YOSHIOKA (Dept. Central Labo., Kansai Med. Univ., Osaka), Isamu SAWARAGI (Dept. Gine., Kansai Med. Univ., Osaka), Tatsuo ABE (Dept. Hygiene, Kyoto Pref. Univ. Med., Kyoto) and Toshiya KOSHIBA (KOSHIBA Clin., Kyoto)

We report a case of chromosome aberration for three generations. The proband, a 30-year-old and normal phenomenal female, has borne a healthy boy and miscarried in twice. At the last abortion, a fetus had 46 chromosomes with a part of unidentified chromosome translocated to the short arms of chromosome 1 and pericentric inversion 9 by aborted chorion. On the examination of her chromosomes, the formal karyotype was 46,XX,t(1,15)(p36.3;q24.3),per inv(9)(p13.3q24.3), on the other hand the chromosome karyotype of her husband was normal. Parents of the proband were examined their chromosomes, father's karyotype was 46,XY,t(1,15)(p36.3;q24.3) and mother's karyotype was 46,XX,per inv(9)(p13.3q24.3). The healthy boy, a first children of the proband, was shown to have the chromosome aberration, it was same karyotype of his grandfather. In our case, the phenotypes and mental faculties were normal. Translocation t(1;15) has been found in only a few previously reported cases. It is moreover uncommon that their chromosome aberration were accompanied with pericentric inversion 9.

C 19

A case of 45,X/46,XYq-/47,XYq-Yq- with ambiguous external genitalia. Takashi UTSUNOMIYA, Masaru SASAKI, Tomoya MUZUNOE, Norio MIHARU, Nobutaka TOYOTA, Katsunori UEDA and Atsushi FUJIWARA (Dept. Obst.& Gynec., Hiroshima Univ. Sch. Med., Hiroshima) Koso OHAMA and Yoshiteru TAKADA (Kure National Hosp., Hiroshima)

A new born infant with a karyotype of 45,X/46,XYq-/47,XYq-Yq- and ambiguous external genitalia was reported. The patient had a penis like phallus and bifid scrotum in the genitalia and a simian line at the left palm. Laparotomy revealed a normal-shaped uterus with a seminiferous tube on the right side and a fallopian tube on the left side. Histological diagnosis of the gonadal tissue was immature testis (right) and ovotestis (left). The infant has undergone clitoplasty and vaginoplasty. Chromosomal analysis of peripheral lymphocytes and fibroblasts of the patient revealed a karyotype of 45,X/46,XYq-/47,XYq-Yq- with a mosaic ratio of approximately 94:4:2. No fluorescent segment was observed on deleted Y chromosomes of the patient. However, the father had a normal Y chromosome with bright segment.

C 20

HOW TO DETECT A TINY INTERSTITIAL DELETION WITHOUT ANY CLINICAL FEATURES SUGGESTING A SPECIFIC CHROMOSOME ABERRATION. Tomiko MOTEGI, Hiroshi HAYAKAWA(Dept. Pediatr., Tokyo Univ. Hosp. Branch, Tokyo)

There have been syndromes with a tiny chromosome deletion, which are associated with a specific clinical feature such as Prader-Willi syndrome and retinoblastoma. In those cases we succeed in detecting a tiny deletion, just paying a special attention to the particular chromosome. Most of the time, however, we have to examine chromosomes of patients whose clinical features do not suggest any specific chromosome aberration, although general features suggest some kind of chromosome aberration. We have experienced two cases of a tiny interstitial deletion without any specific clinical manifestations suggesting a specific chromosome aberration. In one case of del(4)(q2600q2700) the deletion could not be detected on the G-banded chromosomes at the 400-band stage, while the deletion could be detected on those at the same band stage in the other case of del(7)(p15.3p21.3).
Chromosome analysis at 400-band stage is unlikely to detect a tiny deletion on a long arm of chromosome such as 4q. We should report the band stage of examined chromosomes.

C 21

染色体自動分析の実用化についての検討：石井ふみ代[1]・松本典子[1]・新平鎮博[2]・藤田弘子[2]（[1]三菱油化ビーシーエル、[2]大阪市立大学児童保健学）. Practical use of a system for automated chromosome analaysis. Fumiyo ISHII[1], Noriko MATUMOTO[1] Shizuhiro NIIHIRA[2], Hiroko FUJITA[2].([1]Mitubishi Yuka BCL Inc.,Kyoto; [2]Dept. Child Health, Osaka City Univ., Osaka)

画像解析コンピュータの進歩により、染色体自動分析装置の性能も向上し、実用化の段階に入ってきた。マジスキャン（Joyce Loebl 社）を試用する機会を得たので、実用化についての検討を、１００症例を対象にして行った。特に、分析機の精度に関する因子を検討した。今回は、一枚の標本の中で１ｃｍ×１ｃｍのエリアのみを対象にした。分析機が分裂像と認識した中で実際に分裂像であるのは（認識率）、８５％であった。また、肉眼で確認した分裂像の中で分析機が検出できたのは（検出率）、７９％であった。分析機が良いと判断した順に分析をすると、１つの核型を作成するのに必要な分裂像の数は、平均６．４であり、また、５つのカウントを行うのに必要な分裂像の数は、平均１０．２であった（ランキング能力）。今回分析できなかった標本は１８枚（カウントのみ６例、あるいは、核型のみ６例、分析できたのも含まれている）であるが、理由は、標本にゴミが多い、分裂像が少ない、染色体数が足りないなどであった。全く分析できないものは６例であった。また、８例の染色体異常の症例が含まれていたが、モザイクも含めて診断可能であった。一日の処理能力は、平均約４症例であった。今後、長い染色体の分析についての検討が必要と思われる。

C 22

COMPARISON OF THE EFFECTS OF COUNTERSTAINING WITH FLUORESCENT OR NON-FLUORESCENT DYES ON ACRIDINE OR QUINOLINE DERIVATIVES TO HUMAN CHROMOSOMES.

Kouici 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 or umbelliferone, dabsyl chloride, calcein, methyl calcein blue, chromomycin A_3 and 4-6-diamidino-2-phenylindole (DAPI) as fluorescent dyes on five acridine or one quinoline derivative fluorescence of human chromosomes. These five acridine drivatives are 9-aminoacridine, di-9-acridylphenylhydrazone, 2-ethoxy-6-diaminoacridine lactate, 3,6-diamino-acridine hydrochloride and monoacridinocyanul. While the one quinoline derivative is bis(2-quinolyl)-1,4-antholyl dihydrazone. The pairing of DAPI with non-fluorescet A-T specific DNA dye methyl green or crystal violet produced greatly enhanced C bands. The pairing of fluorescent G-C specific DNA dye chromomycin A_3 with 9-aminoacridine or umbelliferone or methyl calcein blue produced R bands. The other pairing of non-fluorescent or fluorescent dyes with fluorescent dyes produced nobands.

C 23

染色体分析における濃淡の検討～クロマトスキャナーの利用：新平鎮博[1)]・藤田弘子[1)]・大内誠司[2)]（[1)]大阪市立大学児童保健学、[2)]大阪市立小児保健センター検査科）。

Investigation of shading pattern of 550 bands human chromosome - utilization of chromatoscanner. Shizuhiro NIIHIRA[1)], Hiroko FUJITA[1)], Seiji OOUCHI[2)]. ([1)]Dept. Child Health, Osaka City Univ.,Osaka ; [2)]Dept. Med. Lab. Children's Medical Center of Osaka City, Osaka)

人の染色体分析に、濃淡を加味して行うと診断精度があがる。その際に用いる５５０バンド濃淡イディオグラムを既に報告しているが、定量性・客観性を高めるために、クロマトスキャナーを用いて検討した。臭化エチジウム添加法による高精度分染の標本で、それぞれ６０本づつの染色体を分析した。そのうち、２から６本が分析可能であった。対象とした黒いバンド２２５バンド中、淡バンド６２、中バンド９１、濃バンド６１本に分類した。既に報告した肉眼で分析したデータと７５％の一致をみた。さらに、濃淡値を細かく分析できるので、同じ、濃淡のバンドも数段階に細かく分類でき、定量性が非常に向上した。ただし、クロマトスキャナーの特徴上、曲がりの強い染色体、重なりの多い染色体の分析には適さないことも分かった。肉眼による分析と併用していく事が必要である。我々の作成した濃淡イディオグラム客観性が、今回の分析で確認できたので、肉眼による分析の際の標準として使用できる。

今回得たデータは、染色体の濃淡分析に用い、染色体診断の精度の向上に役立つ事と、今後進められる染色体自動分析機の重要なパラメータとして利用できる事が示唆された。

C 24

DELETIONS IN THE DMD LOCUS IN DMD AND BMD PATIENTS. Jun-ichi FURUYAMA, Yoshihiro YAMAMOTO, Masafumi HANDA, Masahisa HAGIWARA, Hiromi SAKAMOTO, Osamu MIKAMI, Kiyoshi NAKAMURA, Miho NAKAI (Dept. Genet., Hyogo Coll. Med., Nishinomiya) and Mieko YOSHIOKA (Dept. Pediat., Kobe City General Hosp., Kobe)

EB virus transformed lymphoblastoid cell lines were established in 10 male and 6 female DMD (Duchenne muscular dystrophy) or BMD (Becker muscular dystrophy) patients and 52 their family members. Cellular DNAs were extracted from about $5x10^7$ cultured cells by SDS-protease K-phenol method. Then those DNAs were cleaved completely with HindIII restriction endonuclease, and were analyzed by Southern blot hybridization using cDMD1-2a and cDMD8 (Koenig et.al. Cell, 50, 509-517, 1987) as probes. No deletion in the DMD locus was detected in 16 patients using cDMD1-2a as a probe. For the probe cDMD8, the lack of the HindIII 10 kb and 7 kb DNA fragments was observed in a male patient P30 and a female patient P600, respectively. When agarose gel electrophoresis was done without ethidium bromide and detected with probe cDMD8, RFLPs in 7 kb and 3.1 kb HindIII fragments were appeared in many DNA samples, which was confirmed in two independent experiments.

C 25

A CASE CUTIS LAXA SYNDROME WITH MUSCLE ATROPHY. Yukihisa MATSUDA, Koji SAMESHIMA, Akihiko KODAMA, Koichiro MIYATA(Dept. Pediatr.,Kagoshima Univ KAGOSHIMA) and Itsuro HIGUCHI, Masanori NAKAGAWA, Mitsuhiro OSAME (3rd Intern.,Kagoshima Univ.,Kagoshima)

The patient was a male infant, noted to have an old-appearing face and loose non-hyperelastic skin at birth. At his admission, at the age of 11 months, he was diagnosed as cutis laxa syndrome by histological study from the skin biopsy. After his discharge, he gradually lost the muscular strength, and showed his Gower's sign on standing. Therefore we had preformed the muscle biopsy two times. On the first muscle biopsy, we found that there was the vacuolar change in the muscle, and there were mild increases of muscle fibers glycogen content and increase of acid phosphatase activity in amount fibers. The second biopsy was performed at his age of 3 years and 7 months. It showed that amylo-1,6-glucosidase was obviously decreased.

As above, it suggested that the muscle atrophy of the patient was closely associated with the decrease of amylo-1,6-glucosidase.

C 26

LINKAGE STUDY OF DOMINANTLY INHERITED OLIVOPONTOCEREBELLAR ATROPHY(OPCA) AND HOLMES' ATAXIA.
Hidenao SASAKI, Kunio TASHIRO (Dep. Neurol., Sch. Med., Hokkaido Univ., Sapporo), Akemi WAKISAKA (Dep. Pathol., Sch. Med., Hokkaido Univ., Sapporo), Michihiro C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo), and Sadami SEKIGUCHI (Hokkaido Red Cross Blood Center, Sapporo)

Both autosomal dominant OPCA and Holmes' ataxia are the progressive neurodegenerative disorders of adulthood with unknown biochemical defects. In order to determine the genetic locus and possible genetic heterogeneity, linkage study was performed in 19 OPCA families comprising 180 individuals with 60 affected patients, and 2 Holmes' ataxia families comprising 39 individuals with 10 affected patients. By using computer program LIPED, linkage of each disorder was analysed to 12 blood groups, 5 red blood cell enzymes, HLA-A, -B, -C, and F13A. No evidences suggesting linkage to these two disorders was obtained in the markers examined, including three 6p markers such as HLA, GLO1, and F13A. Furthermore, in 14 out of 15 HLA-informative OPCA families, negative lod scores for OPCA with HLA were obtained at most recombination fractions. Our results provide further evidence suggesting the genetic heterogeneity of dominantly inherited OPCA.

C 27

TWO-DIMENSIONAL ELECTROPHORESIS OF FIBROBLAST PROTEINS IN HUMAN GENETIC DISEASES
Rei-Cheng YANG, Akihiro OSHIMA, Yoshiro NAGAO, Yoshiyuki SUZUKI (Div. Inher. Metab. Dis., Natl. Inst. Neuroci., N.C.N.P., Tokyo)

Recently, two-dimensional electrophoresis (2DE) has become a powerful tool for analysis of a large number of proteins in human cells in physiological and pathological states. We tried to establish a screening system for detecting protein abnormalities in cultured human skin fibroblasts using 2DE and semiautomatic computer analysis. In this study, various pathological cell lines were analyzed; 5 with so-called achondroplasia, 2 with von Recklinghausen disease, 1 with sialidosis, 1 with mental retardation of unknown etiology, and 9 with no known diseases (controls). Quantitative abnormalities were observed in some spots of von Recklinghausen disease and so-called achondroplasia cells, and a new spot was found in a cell line from a patient with the diagnosis of achondroplasia. There was a quantitative difference in one spot between males and females, but no definite changes were detected among different age groups.

C 28

GENETIC POLYMORPHISM OF C82 IN THE JAPANESE POPULATION. Shigeki NAKAMURA, Osamu OHUE and Akiko SAWAGUCHI (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Polyacrylamide gel isoelectric focusing (PAGIEF) of EDTA plasma samples at pH 3.5-9.5 with 3.0M urea followed by semi-dry horizontal electroblotting with enzyme immunoassay was done for the detection of C82 allotypes in 320 unrelated Japanese blood donors living in Tokyo. By using those method, two distinct regions of immunologically detectable C8 were appeared. The C82 polymorphism was detected in the basic region. In 320 Japanese subjects three common and one rare allotypes were observed, and these were considered to be controlled by two common alleles, C82*A and C82*B, and one rare allele which was tentatively designated C82*A2. Family data were in accordance with the Mendelian inheritance. The allele frequencies were estimated as C82*A=0.0422, C82*B=0.9531, and C82*A2=0.0047, respectively. The allele frequencies of C82 in the Japanese population agreed approximately with other ethnic groups. The linkage analyses between the locus for C81 and PGM1 was performed by using LIPED computer program. Rogde et al. (1986) and Rittner et al. (1986) proposed a linkage between C81 and PGM1. In the present study, no evidence for close linkage between C81 and PGM1 was found, although the weak positive lod scores were obtained at all values of the recombination fractions.

C 29

IDENTIFICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN THE HUMAN PYRUVATE DEHYDROGENASE α SUBUNIT GENE. Kiyoshi HASEGAWA, Shigeaki MIYABAYASHI, Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai), Kuniaki NARISAWA (Dept. Biochem. Genet., Tohoku Univ., Sendai) and Shigeo OHTA (Dept. Biochem., Jichi Medical School, Tochigi)

The pyruvate dehydrogenase (PDH) complex occupies a key metabolic position in mammalian cells. Its deficiency in man has been known to cause primary lactic acidosis and variable neurological symptoms. PDH consists of four subunit proteins ($\alpha_2\beta_2$), and the α subunit is important because it contains regulatory sites of the activity of the whole PDH complex. We analyzed genomic DNA from 29 unrelated Japanese individuals by Southern blot hybridization using the human α subunit cDNA as a probe. Restriction fragment length polymorphism (RFLP) was identified at the 10 and 6.2 kilobase pair (kbp) fragments with MspI digestion. The frequencies were 0.33 and 0.67 for the two fragments, respectively. No other RFLPs were detected with the restriction enzymes BamHI, EcoRI, HindIII, PstI, or TaqI. In seven patients with PDH deficiency, no apparent deletions or insertions were observed with MspI or TaqI digestion. There seemed to be the possibility of prenatal diagnosis in one of the three families analyzed because the proband was homozygous for the 10 kbp band and her parents were heterozygotes for the two polymorphic MspI fragments.

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ANALYSIS OF X-LINKED RETINITIS PIGMENTOSA USING A HUMAN ORNITHINE AMINO TRANSFERASE cDNA PROBE. Keiko FUJIKI[1], Yoshihiro HOTTA[2], R.G.WELEBER[3], W.H. MURPHEY[3], M. LITT[3] Mutsuko HAYAKAWA[1], Akira NAKAJIMA[1] & George INANA[2] ([1]Dept, Ophthalmol., Juntendo Univ., Tokyo,[2]National Eye Institute, NIH, Bethesda MD and [3]Dept. Ophthalmol., Med. Genet. and Biochem., Oregon Health Sci. Univ., Portland, OR)

Ornithine aminotransferase (OAT) is a mitochondrial enzyme present in high consentration in the retina, retinal pigment epithelium, and ciliary body. A deficiency of OAT has been demonstrated in patients with gyrate atrophy(GA), a rare hereditary degeneration of choroid and retina that leads to blindness. A cDNA for the human OAT has been constructed and characterized, and the functional OAT gene which is defective in GA and OAT related gene sequences were mapped to chromosome 10q26 and Xp11.2, respectively(PNAS 83:1203-;1986; Invest Ophthalmol Vis Sci 28:1037-;1987). In order to investigate the possibility that the X chromosome genes of the OAT gene family may be linked to or involved in X-linked retinitis pigmentosa(XLRP), we performed genomic Southern analyses of pedigrees with XLRP using a human OAT cDNA as molecular probe.

Several restriction fragment length polymorphisms(RFLPs) were identified, and Msp I RFLP of 3.8 kbp OAT X chromosome fragment was associated with XLRP.

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THE CEREBRO-OCULO-FACIO-SKELETAL(COFS) SYNDROME. Ryuhei KODAKA, Masahisa FUNATO, Seiichi SHIMADA, Hiroshi TAMAI, Hideo TAKI, Yasushi YOSHIOKA, Kazuko NOMA, Yasuko KINOSHITA(Dept. Pediatr., Yodogawa Christian Hosp., Osaka) Hideaki DEJIMA(Dept. Perinatol., National Cardiovascular Center, Osaka) Hideaki CHIYO(Daito Health Care Center, Osaka)

The COFS syndrome was first described by Pena & Shokeir in 1974 as an autosomal recessively inherited multiple congenital anomaly syndrome, and since then 29 cases have been reported. We report a further case. -Case report- This 8-month-old male infant was born by spontaneous delivery at term, to a gravida-1, para-1, 23-year-old Japanese mother and a 23-year-old American father, weighing 1633g, with head circumferance of 26.0cm, and with Apgar score 1(1min), 3(5min), 5(10min). He has typical features of this syndrome; i.e., microcephaly, cataracta, prominent nasal root, low-set & large ear pinna, hypognathia, short neck, scoliosis,flexion joint contracture, camptodactyly, coxa valga,virtical talus,rocker-bottom appearance of feet, posteriorly placed second metatarsal bone,osteoporosis,hypotonia, and failure to thrive. Additionaly, he has partial agenesis of corpus callosum,cavernous hemangioma of lip & neck, and laryngomalasia. Chromosome analysis showed 46, XY, 13-p satellite as a normal variant, which chromosome type was also detected in his mother. There was no remarkable abnormal finding in laboratory data including TORCH-titers.

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POLYMORPHISM OF FXIIIA SUBTYPE AMONG VARIOUS MONGOLOID AND CAUCASOID POPULATIONS. Koichi SUZIKI, Kiyoshi MATSUI, Kiyoshi FUJITA, Hideo MATSUMOTO (Dept. Legal Medicine, Osaka Med. College, Osaka) and H.W. GOEDDE (Institute of Hum. Genet., Univ. of Hamburg, Hamburg)

The subtypes of the A subunit of the coagulation factor XIII (FXIIIA) were investigated on 17 populations from Asia and Europe. The subtypes were first revealed by isoelectric focusing in polyacrylamide gels (PAG) containing 2M urea with narrow pH range 5-6.5. In this study PAG was substituted for agarose gels. It was found that FXIIIA subtypes occur ubiquitously in all the populations tested. FXIIIA*1B occurs most frequently among the populations. FXIIIA*1A is more frequent in Mongoloid populations than in Caucasoid populations but in the other hand FXIIIA*2B is more frequent in Caucasoid populations than in Mongoloid populations. Four new variants were suspected among the populations of Japanese, Thailanders, Indians and Jews. They were tentatively named FXIIIA*11, 12, 13, and 14 according to the distance from the anode.

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Genetic markers of squirrel monkeys.
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Genetic variability of squirrel monkeys (Saimiri sciureus), one of new world monkey species, was surveyed using blood samples of 44 wild origin individuals. Patterns of red cell enzymes, ACP, ADA, ESD, PGD and PGM, were detected by starch gel electrophoresis. Gc, a serum protein, was detected by isoelectro-focusing. Among these proteins, only PGM was highly polymorphic and four phenotypes, 1, 2-1, 2 and 3-1, were observed. The family study on the wild origin parents and their children showed that the PGM polymorphism was genetic. Allele frequencies in the wild origin population were, 0.68 for PGM-1, 0.31 for PGM-2 and 0.01 for PGM-3. As for ESD, one individual had a different pattern. No variant could be found in the other four proteins. Restriction enzymes' digestion patterns of mitochondrial DNA were detected by Southern hybridization method using total DNA extracted from blood cells. One individual out of 15 surveyed so far showed a variant AvaII digestion pattern. There was no variation in BamHI, HincII, HindIII and SstI digestion patterns of mitochondrial DNA among the 15 individuals.

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PATTERN FORMATION AND MORPHOLOGY OF VOLAR PADS: EMBRYOLOGICAL OBSERVATION OF INBRED STRAINS OF RAT WITH DIFFERENT DERMATOGLYPHIC PATTERNS. Michio OKAJIMA (Dept. Forens. Med., Tokyo Med. Dent. Univ., Tokyo)

The manifestation of dermatoglyphic configurations is influenced by genetic and environmental factors. It has been generally believed from comparative observation of volar pads of human and non-human primates that the arrangement of skin ridges is related to the morphological nature of the volar pad during the period of ridge differentiation. However, this hypothesis has not yet been unequivocally proved. Recently, the present author discovered the existence of dermatoglyphic traits in the rat, each inbred strain possessing disinctive patterns. In the present investigation, volar pads were studied in fetuses at gestational days 20 and 21 in two inbred strains of rat, NIG and ACI, and in their F_1 hybrids. The pads of fetuses from the NIG strain showing whorl patterns presented a conical and erect contour, the core of the pattern being located at the summit of the pad. On the other hand, pads of fetuses from the ACI strain showing triradial patterns exhibited suppressed elevation with an extended contour. The pads of F_1 hybrids were intermediate in form between the two parental strains. These experimental observations support the above hypothesis.