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Abstracts of General Contribution, the 32nd Annual Meeting of the Japan Society of Human Genetics

A 1

PROXIMAL PARTIAL MONOSOMY OF THE LONG ARM OF CHROMOSOME 4 WITH CONGENITAL LEUKODERMA AND SCALP APLASIA CUTIS. Keiko WAKUI, Norishige YAMAMOTO (Dept. Clin. Lab., SCMC, Saitama), Atsuo YOSHINO, Hiroshi NISHIMOTO (Dept. Neurosurg., SCMC), and Yoshifumi YAMAMOTO (Dept. Ped. Jichi Med. Sch. Tochigi and Dept. Med. Genet., SCMC).

Among the various partial monosomies of 4q, distal ones(4q31→ter) have been regarded as a recognizable syndrome. We report a male infant with proximal monosomy of the long arm of chromosome 4 [46,XY, del(4)(q12q21.1),de novo]. The clinical features of the patient included congenital partial leukoderma, scalp vertex aplasia cutis, agenesis of the right kidney, psychomotor retardation, flat nasal root , micrognathia and wide spaced nipples.
Previously, only 7 cases with proximal 4q monosomy including 4q13 have been reported. Among these cases, three patients have been associated with autosomal dominant piebald trait. Although white skin was limited only over the lower abdomen and lower legs, the condition of low expressivity of this trait can be considered in our patient.
Scalp aplasia cutis has not been described in previous reports.
Gc-system typing by isoelectric focusing in polyacrylamide gels revealad a father-child incompatibility and suggested that the locus should be on segment 4q12-q13.

A 2

CASE RERORT OF de novo 4p TRISOMY.
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A case of de novo 4p trisomy was reported. A 10-month-old female patient was born to 28 year-old mother and 36 year-old father as a second child at full term with the birth weight of 3300g. The parents had no history of abortion and were non-consanguineous. Her elder sister was healthy. Her mother had severe vomiting and imminent abortion at her 2nd gestational month. After birth, she showed muscular hypotonia and obstructive respiration with stridor. She had an asymmetric skull, broadbridge nose, antimongoloid folds, mild exophthalmos, high arched palate, short neck and camptodactyly. On her finger print, she had five whorls and one double whorl. She showed severe mental and physical retardation with seizure and deafness. Chromosomal analysis with high resolution banding revealed that the patient's karyotype was 46,XX,inv dup(4)(p14→16). Since the chromosome constitution of her parents was normal, 4p trisomy seen in the patient was considered to occur de novo.

A 3

PARTIAL TRISOMY 9q IITH AN ATYPICAL PHENOTYPE: A SUBCLASSIFICATION OF 9q PARTIAL TRISOMY. Yoshifumi YAMAMOTO(Dept.Ped.Jichi Med.Sch.,Tochigi and Dept.Med.Genet.,SCMC,Saitama),Noriko OKAMOTO(Dept.Ped.Jichi Med. Sch.),Takahiro NARA,Hideki HORITA(Dept.Neurol.,SCMC),Naoki Niitsu(Dept. Neonatol.,SCMC) and Satohiko IMAIZUMI(Dept.Surg.,SCMC).

Among partial trisomies of 9q, distal ones have been regarded as a readily definable syndrome. We report a male infant with trisomy of the long arm of chromosome 9[46,XY,-12,+der(12)inv ins(12;9)(p13;q32 q13)]. The clinical features of the child which included SFD, developmental retardation, pyloric stenosis, inguinal hernia, retentio testis, muscle hypertonia, broad nose, flat philtrum, thin lips, micrognathia, malformed ears and abnormal fingergrasping without contracture, were not similar to those of distal 9q trisomy. Based on analysis of previously reported cases with various duplicated segments, we can subdivide partial 9q trisomies into 1) distal 9q(9q34～qter) trisomy with narrow and long face, profound thin and long extremities and arachnodactyly with contracture, 2)9q32 trisomy characterized by beaked nose and tapering fingers, and 3) proximal 9q trisomy with unspecified clinical features.
The frequency of pyloric stenosis is relatively high(57%) among cases with proximal 9q(9q32～)trisomy. The proximal segments appear to have some influence on the development of pyloric stenosis.

A 4

A CASE OF 21 TETRASOMY. Hiromi SAKAMOTO, Masahisa HAGIWARA, Yoshihiro YAMAMOTO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med., Nishinomiya), Aiichiro TANAKA, Takakuni TANIZAWA, Hiroyoshi WADA (Dept. Pediat., Hyogo Coll. Med., Nishinomiya), Yoshie SUGAWARA and Hiroko MIMURA (Dept. Clin. Lab., Hyogo Coll. Med., Nishinomiya)

A 9-month-old girl was referred to us because of developmental delay and odd looking face. She was the first child of a 33-year-old father and a 38-year-old mother. She was born after an uneventful gestation period of 41 weeks and 2 days. Her birth weight was 2814 gr. Her palpebral fissures were slanted upward and outerward. She showed epicanthus, hypertelorism, low nasal bridge, antiverted nostril and low-set ears. Her fingers were short and thick. She could hold her head at the age of 4 months. She could not sit alone. Cytogenetical examination revealed an additional marker chromosome. The marker chromosome was examined using banding methods: GTG, QFQ, high resolution GTG, CBG, NOR, DA-DAPI and RBG. Her parents' karyotypes were normal. We supposed that the marker chromosome was derived from two chromosomes 21. The red blood cells SOD1 activity was within normal range, so the marker chromosome did not contain the locus for SOD1 gene. We concluded that her karyotype was 47,XX,+psu dic(21)t(21;21)(q22.1; q22.1).

A 5

GENE DOSAGE STUDY OF DIAPHORASE 1 (DIA1) AND ARYLSUPHATASE A (ARSA) IN A CASE WITH A TERMINAL DELETION OF THE LONG ARM OF CHROMOSOME 22.

Yukio TAKAHASHI, Kouji NARAHARA, Masae MURAKAMI, Kei HIRAMOTO, Tsunenori MATSUBARA and Hiroshi KIMOTO (Dept. Pediatr., Okayama Univ., Okayama)

In Human Gene Mapping 8, DIA1 and ARSA have been mapped to 22q13.31→qter. We studied gene dosage effects for DIA1, ARSA, alpha-L-iduronidase (IDUA), alpha-galactosidase B (NAGA) and beta-galactosidase (GLB2) in a case with a deletion of the long arm of chromosome 22. High resolution banding analysis showed the karyotype of 46,XX,del(22)(pter→q13.31:) de novo. Assays of the enzymes from red blood cells and lymphoid cell line established by EB virus revealed that ARSA activity was reduced to a half of normal values (52 % of the normal and 43 % of the mean parental value), while activities of the remaining enzymes were all within the normal range. These results suggested the localization of DIA1 in the proximal 22q13.31 region. In addition, if a gene-dosage relationship exists in NAGA and GLB2, these two loci may be excluded from 22q13.31→qter.

A 6

SPORADIC RECURRENT REARRANGEMENT IN ROUTINE CYTOGENETIC ANALYSIS.

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From 1984 to 1987 we have observed about 6000 trypsin-Giemsa banded metaphases in PHA stumulated lymphocytes from 323 patients, among which a total six cells seem to have a de novo rearrangement. Two particular rearrangemet t(7;14)(p14.2;q11.2) in two cells and inv(14)(q11.2q32.3) in four cells were present. These six cells were noted in six different patients, studied for disparate reasons; micropenis with 46,XY, congenital hart disease (PDA) with 46,XY, psychomotor retardation with 46,XY, failure to thrive with mos 46,XX/46,XX,t(2;4)(q37;q21), psychomotor retardation with 46,XX,inv(1)(p13q21),-21, +der(21),t(9;21)(p12?;p11) and primary amenorrhea with 46,X,t(X;9)(q21.32;q22.31). None was known to have a chromosome instability syndrome, malignancy, or unusual X-ray or drug exposure. The breakpoints of these rearrangements appeared to reside near sites of gene involved in lymphocyte differention.

A 7

A family with dentatorubropallidoluysian atrophy (DRPLA), protuberance in the median portion of hard palate, cavum septi pellucidi, cavum Vergae, and / or abnormal chromosome (15p+).

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The proband was a 17-year-old boy born from consanguineous parents. At age 16 he became unsteady in gait and complained of involuntary movement of his neck and head. Slurred speech, myoclonus on neck and shoulders, and ataxic gait were noticed. Romberg's sign was negative. The brain CT revealed cerebellar atrophy. His sister aged 24 had similar clinical manifestations. They were diagnosed as DRPLA because of their signs and symptoms. In addition, they had protuberances in the median portion of hard palate and abnormalities in ventricular system such as cavum septi pellucidi and cavum Vergae. Their chromosomes showed 15p+. Their father had also the protuberance and 15p+, but no abnormality in the ventricle and involuntary movement. Their mother had only the abnormality in ventricle. Their younger brother aged 10 had 15p+ and the abnormal ventricle. These results suggest that both the protuberance and 15p+ are dominantly inherited from the father to his 2 children and abnormality in ventricular system is dominantly inherited from the mother to her 3 children. DRPLA in this family appears to be inherited as an autosomal recessive trait.

A 8

A FEMALE CASE OF ANHIDROTIC ECTODERMAL DYSPLASIA WITH DE NOVO X/15 TRANSLOCATION.

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We report here a sporadic female case with ectodermal dysplasia,anhidrotic (EDA) having a X/15 translocation chromosome.

The patient was a 2 months old girl. The body weight was 2665 g at her birth after full term pregnancy. She was a second child of healthy parents who had no consanguinity. The patient showed hypohidrosis, hypotrichosis, dental anomalies, dry skin and saddle nose. Due to these features, she was diagnosed as EDA. There was no family history about this syndrome in this case. Chromosome studies were performed on the peripheral blood cells and skin fibroblasts obtained from the patient and her family. The results showed that she had de novo X/15 translocation and normal X chromosome was preferentially inactivated. The break point of the X chromosome was at the band q12 or near region. This was not inconsistent with the results of the previously reported female EDA with t(X;9) and the regional localization of the EDA gene performed by molecular genetic studies.

A 9

CYTOGENETIC FEATURE IN A NEW VARIANT OF CHROMOSOME 16.

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A number of chromosomal polymorhisms which are positively stained by C- and Q-banding techniquces and also the differences in amount or position of the constitutive heterochromatin does not influence for the phenotypes have been recognised in human chromosomes. Recently, however, new variant of chromosome 9 and 16 have been reported with C-band negative and G-band positive material in the proximal region of the short arm. We have been experienced 16p+ which has a excessive segment in the proximal region of the short arm in 22 cases of chromosome studies for 1986 to 1987(15315 cases). In this excessive segment of chromosome 16, some cytogenetic feature were established as following results: 1) This excessive segment are detected as G-band positive but C- and DA/DAPI band negative material in the proximal region of short arm. 2) This excessive segment have variable size. 3) Chromosomal abberations are never found in other autosome or sex chromosome. 4) This chromosome are found in defferent kindreds and are transmitted as simple Mendelian trait. 5) Any common clinical feature could not find in those probands.

In conclusion, it seems that this excessive segment does not include any structural gene or, even if it includes, this structural gene any functionally under inactivation. Therefore, this excessive segment does not produce phenotypic effects in the carriers and this is one of the variant of chromosome.

A 10

Interstitial Deletion of the Long Arm of Chromosome 16: A Proposal that Band q22 is Critical for 16q Deletion Syndrome Kenji Naritomi, Chuken Miyagi, Yosinori Izumikawa and Kiyotake Hirayama. Department of Pediatrics, School of Medicine, University of the Ryukyus, Okinawa, Japan.

Deletions of the long arm of chromosome 16 are extremely uncommon. Though ten cases had been reported in the literature, the critical band region was conflicting.

The propositus was a 2-month-old female born to healthy 27 y/o mother and 33 y/o father. They are not related. She was delivered at 36th week of gestation after uneventful pregnancy. Birth weight was 2,135 g. Physical findings were profoud growth and psychomotor retardation, meningoencephalocele, mild hydrocephalus, large anterior and posterior fontanels, diastasis of cranial sutures, high forehead, flat and broad nasal bridge, low-set posteriorly rotated ears, high-arched palate, short neck, widely set nipples, digital pads, broad great toes, medially curved toes and hypotonia. Chromosomes analyzed with high resolution GTG and RBG banding techniques revealed a karyotype:46, XX,del(16)(q21q23). The parental karyotypes were normal.

Delineation of 16q deletion synfome was supported by a summarization of clinical and cytogenetical findings of eleven cases. As a result of a comparison of the deleted band regions among them, it was concluded that critical band region of 16q deletion syndrome is q22.

A 11

THE ROLE OF EXTRA CHROMOSOME 21 MOSAICISM IN THE OCCURRENCE OF TRANSIENT ABNORMAL MYELOPOIESIS (TAM). Kyohko ABE, Sei OKIMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki), Masanori YANAI (Dept. Pediat., Nagasaki Univ.), Hiroyuki TSUCHIYA (Dept. Pediat., Kumamoto Univ., Kumamoto), Naoki HARADA (Kyushu Med. Sci. Chrom. Labo., Fukuoka)

TAM, which resembles congenital leukemia but spontaneously resolves, sometimes occurs in Down syndrome newborns (DS) and rarely in phenotypically normal babies (NDS). We report 2 NDS and 1 DS with TAM, and 1 NDS with either TAM or leukemia. Chromosome analyses were performed in the bone marrow (BM) cells, peripheral blood lymphocytes and skin fibroblasts each at the TAM and the remission period. Independent of DS or NDS, at the TAM period, all patients had a 46/47,+21 or a 47,+21/48,+21,+21 mosaicism in BM, and the cell line having extra #21(s) selectively increased. The origin of extra #21 was studied using QFQ- and RFA heteromorphisms as markers. The cell line with extra #21 in one patient originated in mitotic non-disjunction before the differentiation for BM tissue during organogenesis, and that in another patient resulted from non-disjunction after the BM tissue differentiation. From the results of the present study, we propose a hypothesis that TAM may occur as a result of unbalanced interaction between the cell with extra # 21(s) and the germ line cell.

A 12

ANNUAL CHANGES IN CHROMOSOMALLY ABNORMAL CLONES IN BONE MARROW OF THOROTRAST DEPOSITED PATIENTS. Takaaki ISHIHARA, Masako MINAMIHISAMATSU (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba) and Sei-ichi KOHNO (Dept. Biol., Toho Univ., Funabashi)

Chromosome abnormalities in bone marrow and peripheral lymphocytes of Thorotrast administered patients have been studied in order to elucidate the relationship of such chromosome abnormalities to the late effects of radiation. Fourteen types of clones with radiation-induced chromosome abnormalities have been observed in 11 of 52 patients. We present the results of annual follow-up examinations in 3 of the 14 clones which occurred with high frequencies. During the several years of observations, the 3 clones showed different manners of proliferation from one another. The incidence of one clone was nearly constant, that of another clone showed a tendency of increase, and still another clone showed drastic changes in frequency from year to year. Large chromosomal losses were specific features common to the 3 clones. Appearance of clones with partial or whole chromosomal losses has often been observed in experimental studies using X-irradiated rats. The problem of the relationship between chromosomal losses and clone formation in bone marrow after irradiation are discussed.

A 13

CYTOGENETIC BASIS IN THE GENESIS OF PRADER-WILLI SYNDROME (PWS). Kei HIRAMOTO, Kouji NARAHARA, Yukio TAKAHASHI, Masae MURAKAMI, Kiyoshi KIKKAWA and Hiroshi KIMOTO (Dept. Pediatr., Okayama Univ., Okayama)

The band 15q11.2 is believed to be the critical monosomic segment for the genesis of PWS (Human Gene Mapping 8). However, in some cases normal karyotype, balanced translocation involving chromosome 15 or tetrasomy for chromosome 15 [inv dup(15)] has been reported. The possibility remains that PWS may result from genetically heterogeneous etiologies. We saw two clinically typical PWS patients with a chromosome abnormality; t(15;15) and +inv dup(15). Cytogenetic studies showed that patient No. 1 had a mosaic karyotype: 45,XX,-15,-15,+t(15;15)(p11.1;q11.2)/46,XX,-15,-15,+t(15;15)(p11.1;q11.2),+r(15)(p11.1::q11.2). The t(15;15) chromosome retained apparently intact q11.2 bands in both arms. Karyotype of patient No. 2 was 47,XX,del(15)(pter→q11.1::q12→qter),+inv dup(15)(pter→q11.1::q11.1→pter), being essentially monosomy for 15q11.2. These results suggested that the cause of PWS can be simply explained by monosomy for the critical segment which is located in the very proximal region of band 15q11.2. Confirmation of this hypothesis has to await a molecular study using cDNA encoding the critical segment in PWS cases with normal karyotypes.

A 14

MLC BLOCKING EFFECT IN SPONTANEOUS ABORTIONS.
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To reveal the role of blocking antibody in spontaneous abortions, mixed lymphocyte culture (MLC) and chromosome analysis of the abortuses were performed. The subjects included 21 patients with spontaneous abortion of unknown etiology and 19 women with uneventful pregnant course (control group). Blocking effect (BE) was detected by MLC (stimulator; husband's lymphocytes, responder; wife's lymphocytes) in serum of the wife, and was expressed as follows. BE = (1-MLC in the serum/MLC in AB serum) X 100 (%). Mean BE of the patients with spontaneous abortion (-8.8%) was significantly lower than that of the control group (21.2%) ($p<0.05$). Out of the 21 abortuses, 9 were found to be chromosomally abnormal. Mean BE of the patients with chromosomally abnormal abortuses (-14.9%) was not significantly different from that with chromosomally normal abortuses (-0.64%). These results suggest that the decreased BE in patients with spontaneous abortion is the result of abortions, rather than the cause of abortions.

A 15

Effect of genes on the break points of balanced chromosome rearrangements: Elimination of homozygotes at the early developmental stages.
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Chinese hamster lines heterozygous for 2 inversions and 15 reciprocal translocations between autosomes were used in the present study. From the crosses between males and females heterozygous for the same rearrangements, these homozygotes were produced in 14 out of 17 lines. In the remaining 3 lines [T(1;3)7Idr, T(1;3)8Idr and I(4)104Idr], however, no homozygote for the rearrangements was obtained. The frequencies of homozygotes expected from MII analysis in T7/+, T8/+ and INV25/+ heterozygotes were 5.6%, 6.2% and 20.3%, respectively. Then, zygotes obtained from the crosses between heterozygotes in these lines were analyzed at early developmental stages. These homozygotes were eliminated during the early preimplantation period or just after implantation. In further cross experiments of T8/+ heterozygotes with other translocation heterozygotes bearing the same translocated segments of chromosomes 1 and 3, offspring with both translocations were obtained. This result indicated a negative evidence for the position effect of genes on the translocated segments in T(1;3)8Idr. These findings, therefore, suggest that the homozygosity of the breakage or deletion of genes on the break points might have influence on early development of embryos.

A 16

IN VIVO ASSAY FOR MEIOTIC NONDISJUNCTION IN FEMALE CHINESE HAMSTERS: EFFECT OF CHEMICAL COMPOUNDS WITH A TRIMETHOXYBENZEN RING.
Hiroyuki TATENO, Yujiroh KAMIGUCHI and Kazuya MIKAMO (Dept. of Biol. Sci., Asahikawa Med. Col., Asahikawa)

Many of inhibitors of tubulin polymerization have a trimethoxybenzen ring. Therefore, chemicals with a such structure may induce meiotic nondisjunction. In this study, podophyllotoxin (20 μg/g b.w.), reserpine (0.5 μg/g b.w.) and trimethoxybenzoic acid (500 μg/g b.w.) were injected intraperitoneally at the onset of formation of the first meiotic spindle in females which maintain normal estrous cycles. The former is an inhibitor of tubulin polymerization, while the latter two are not known yet as the inhibitor. Incidence of secondary oocytes with a giant polar body increased significantly ($P<0.01$) by the treatment with podophyllotoxin (16.4%;28/171) and reserpine (8.9%; 18/202) as compared with the control (1.4%; 3/212). Chromosome analysis of secondary oocytes revealed that only podophyllotoxin was effective in inducing of aneuploids (40.0%; 62/155 vs control 1.5%; 3/198) as the result of nondisjunction or anaphase lagging. These phenomena occurred frequently in abnormal oocytes with a large polar body. Our experiment showed that podophyllotoxin was capable of inducing both nondisjunction and anaphase lagging very likely owing to the inhibition of tubulin polymerization, but reserpine and trimethoxybenzoic acid seemed not to have such an ability.

A 17

EFFECTS OF BrdU ON FORMATION OF SCES AND CHROMOSOMAL ABERRATIONS IN BLOOM SYNDROME FIBROBLASTS. Hideo TSUJI (Div. Genet., Natl. Inst. Radiol. Sci., Chiba), M.W. HEARTLEIN, S.A. LATT (Children's Hosp., Boston)

It has not been conclusive that Bloom syndrome (BS) has a high incidence of spontaneous SCEs, because BrdU used to visualize SCEs has an inducible effect on SCEs. We examined the effect of BrdU on the induction of SCEs and chromosomal aberrations (CAs) in BS fibroblasts in the range of 1 to 100% BrdU substitution for Thd in DNA. SCEs were detected by an immunofluorescent method with anti-BrdU antibody. Six BS fibroblasts exhibited a constant SCE frequency (40/cell) below 5% BrdU substitution (8 times that of normal cells). Above 10% BrdU substitution, SCE frequency increased linearly with increasing BrdU substitution; BrdU-induced SCEs were 6 to 10 times higher in BS cells than in normal cells. Analysis of SCEs occurring during three cell cycles revealed that SCE frequency is constant among cell cycles at 1% BrdU substitution whereas at 100% substitution, SCEs were induced in second and third cycles. The results indicate that BS fibroblasts are highly sensitive for SCE induction to BrdU incorporated into template DNA at high substitution levels whereas at low substitution levels BrdU effect is minimal, implying that spontaneous SCEs are abundant in BS cells. On the other hand, BrdU-induced CAs were only slightly higher in BS cells than in normal cells, suggesting that BrdU effect for BS cells is differrent between SCE induction and CA induction.

A 18

FRAGILE SITES ON FIBROBLASTS FROM HYDATIDIFORM MOLE AND NORMAL HUMAN SKIN. Ichiro MURANO, Akira KUWANO and Tadashi KAJII (Dept. Pediat., Yamaguchi Univ. Sch. Med. Ube)

Three common fragile sites, 1p31, 3q26, and 7q11.2, were most frequent in skin fibroblasts from three normal individuals cultured with 0.2μM aphidicolin for the last 26 h, while those at 3p14, 7q32, 14q24, 16q23 and Xp22 were most frequent in the PB lymphocytes cultured under the same condition. Fibroblasts from two XX complete moles resulting from duplication of a haploid sperm exhibited breaks in the order of 1p31, 7q31, 9p21 and 16q23. Chromatid breaks took 91% of breaks in normal fibroblasts, 55% of PB lymphocytes and 66% of molar fibroblasts. Cells were also treated with a combination of 10^{-7}M 5-fluorodeoxyuridine for 24 h and 2.2mM caffeine for 6 h, giving similar results.

A 19

CHROMOSOMAL FRAGILE SITES IN PATIENTS WITH BENIGN AND MALIGNANT TUMORS. Motoi MURATA, Mikako OTSUKA (Div. Epidemiol., Chiba Cancer Center, Chiba), Ei-ichi TAKAHASHI and Tada-aki HORI (Div. Genet., Natl Inst. Radiol. Sci., Chiba)

Etiological association of chromosomal fragile sites with human cancer has not fully been clarified. We conducted a survey study of heritable rare fragile sites in patients (121 males and 146 females) who were admitted to Chiba Cancer Center Hospital. They consisted of 78 digestive tract, 17 lung, 27 gynaecologic and 93 other cancers and 52 benign diseases including myoma uteri, ovarian cyst, cervical dysplasia, etc. Chromosomes in cultured lymphocytes were tested for folate sensitive, distamycin A-inducible and BrdU-requiring fragile sites. Among all patients, 14 carriers were found. They were all of the distamycin A-inducible type, i.e. fra(8)(q24), fra(16)(q22) and fra(17)(p12). One patient with uterine cervical cancer was a double carrier of fra(16)(q-22) and fra(17)(p12). As a whole the incidence, 5.6%, was in good agreement with that observed in the general population. If the whole subjects were classified by their diseases and ages, then those with gynaecologic benign and malignant tumors showed a significantly higher rate (11.1%) than the others (4.2%). The elevated rate was also seen in younger than older age groups. We consider that this chromosomal instability is associated at least with some specific malignancies.

A 20

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

The chromosomal fragility of the lymphocytes from 100 patients, undergoing surgery for the lung tumor was studied. Two patients (pts) had double primary cancers and the pathological diagnosis of the excised tumor were as follows; 40 adenocarcinomas (AD), 2 adenosquamous cell ca., 28 squamous cell ca. (SQ), 9 large cell ca., 3 small cell ca., 2 undifferentiated ca., 2 carcinoid tumor, 1 mucoepidermoid tumor, 8 metastatic cancers, 1 pseudolymphoma and 6 benign tumors. 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). Eleven carriers of rare fragile sites (FS) were found; distamycin A inducible fra(17)(p12) were detected in 3 pts with AD, 1 with carcinoid tumor and 1 with pseudolymphoma, distamycin A inducible fra(16)(q22) in 2 pts with SQ and 1 with AD, folate sensitive fra(11)(q13) in a pt with SQ, folate sensitive fra(16)(p12) in a pt with AD and BrdU requiring fra(10)(q25) was seen in 18 year old girl with bronchiectasis. Thus the frequency of rare FS in the primary lung cancers was 10.6%, revealing higher than normal control (5.2% by Takahashi et al.).

A 21

AUDITORY BRAINSTEM RESPONSES IN THE FRAGILE X SYNDROME. Tadao ARINAMI, Miki SATO (Ibaraki Prefectural Colony Hosp.), and Ikuko KONDO (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Auditory brainstem responses were recorded from a group of 12 mentally retarded male adults with the fragile X syndrome and compared with those from a control group of age-matched male adults with normal intelligence. The responses were analyzed in terms of ABR thresholds, absolute latencies, interpeak latencies. One patient had an increased ABR threshold of 60dBnHL, indicating hearing impairment. Statistical analyses of the data excluding those of this patient showed significantly longer wave V latency and III-V interpeak latency at 70dBnHL stimuli for the fra(X) group, but no difference between groups for I-III interpeak latency. This pattern of interwave latency differences suggests possible differential CNS involvement in the fragile X syndrome.

A 22

FREQUENCY OF FRAGILE X SYNDROME IN A POPULATION WITH INFANTILE AUTISM. Tadao ARINAMI (Ibaraki Prefectural Colony Hospital, Ibaraki), Youko TANOUE (Yuhara Hosp., Ibaraki) and Ikuko KONDO (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

To evaluate an importance of fra(X) as a genetic etiology of infantile autism, we screened for fra(X) in 106 subjects with autism (85 males and 21 females) from 104 families, ranging in age from 2 to 30 years with a mean age of 11 years. Most of them were outpatients and living at home. The diagnoses of autism were made using DSM-III criteria. Nine cases from seven families among the subjects had a family history with autism/mental retardation. Two methods of fra(X) induction with MTX and FUdR were used. One boy aged three with moderate intellectual impairment was found to have a fragile X chromosome. One of his maternal cousins and two of his maternal aunts were mildly mentally retarded. In the course of treatment with a special educational program for him, his autistic characteristics constituting Kanner's two main criteria for autism, namely lack of affective contact and resistance to change, was fairly improving. There was, however, no improvement in language delay, shyness and hyperactivity. We suppose that autistic-like behavior some fra(X) carriers show is due to a combination of delay in social relationships, extreme shyness and distractibility.

A 23

PREVALENCE OF CHROMOSOME ANOMALIES IN MENTALLY RETARDED SCHOOL CHILDREN: STUDY OF POPULATION INCIDENCE OF THE FRAGILE X SYNDROME.
Ikuko KONDO[1] and Tadao ARINAMI[2] ([1] Dept. of Hum. Genet., Univ. of Tsukuba and [2] Ibaraki Prefectural Colony, Ibaraki)

We have studied survey of 1500 mentally retarded children who are attending in 13 special education schools in Ibaraki to determine the prevalence of chromosome anomalies, specially of the fragile X syndrome(fra(X)). A letter explaining the purpose of the study was mailed to parents together with a questionnaire which included 17 questions. The questionnaire was filled in and returned by 894 parents, giving a responce rate of 60%. One hundred of sixty seven children(18.7%) had chromosome analyses before and 95 (8.7%) had chromosome anomalies: Most common one was Down syndrome, but fra(X) was not dianosed. Family history of mental retardation was present in 32(3.6%) and typical phenotypes of the fra(X) were filled in 53 (5.9%). These data indicate that the fra(X) may be a second common mental retardation in special education school children. Now, we are carring out chromosome analysis in the children with family history and/or phenotypes of the fra(X) to confirm the fragile X chromosome and to examine the usefulness of the questionnaire to diagnose clinically this syndrome.

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

Since fragile sites (FS) are suspected to induce instability of genomes, population studies should be needed to investigate the clinical implication of FS. Then, we have carried out a survey of FS for 288 Japanese institutionalized mentally retarded patients. The purposes of the present study are to investigate the frequency of patients with FS on X chromosome (fra X syndrome) in a mentally retarded Japanese population and to compare the frequency of autosomal FS (AFS) between karyotypically normal and abnormal patients. Blood samples from the patients were cultured in TdR and folic acid free Ham's F10 medium or treated with 50 μg/ml distamycin A or 7 μg/ml BrdU. When the frequency of chromatid aberrations on a certain locus were more than 4 %, it was regarded as FS. 12 male patients with fra X syndrome were found in 165 karyotypically normal males (7.3 %). Carriers of AFS in 221 karyotypically normal and 55 karyotypically abnormal patients were 11 (5.0 %) and 9 (16.4 %), respectively. The present result supports the view that AFS can induce instability of genomes.

A 25

NEWLY FOUND RARE FRAGILE SITES AT 8q24.1 AND 16p12.1: INHERITANCE AND THE MODE OF EXPRESSION. Ei-ichi TAKAHASHI, Tada-aki HORI, Masako MINAMI-HISAMATSU, Takaaki ISHIHARA (Div. Genet., Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba), Kyoko HIMI, Masako ANDO (Dept. Med., Chiba Univ., Chiba) and Motoi MURATA (Div. Epidemiol., Chiba Cancer Center, Chiba)

It has been suggested that fragile sites (fra) may act as predisposing factors for genomic instability and may be associated with a variety of clinical manifestations. We examined the inheritance and the mode of expressions of newly found fragile sites at 8q24.1 and 16p12.1. Two families (7 subjects) for fra(8)(q24.1) and one (5 subjects) for fra(16)(p12.1) were examined. Each site was inherited to the kindreds in a Mendelian codominant fashion. The expressions of both sites were induced by AT-specific ligands, distamycin A, Hoechst 33258, berenil and DAPI. Triradials were also found. These fragile sites were located at the interface between G- and R-positive bands. The incidences of fra(8)(q24.1) and fra(16)(p12.1) in a Japanese population were 0.71% and 0.12%, respectively. They can be classified as rare heritable distamycin A-inducible fragile sites. They may be the sites which are endemic to the Japanese subjects.

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

Clinical features including psychological findings were investigated on 11 carriers with fragile X(FX) and autosomal fragile sites(AFS) screened from 225 mentally retarded Japanese patients. The ages of them with FX and AFS ranged from 18 to 48 and 14 to 58 years, respectively. Mean IQ of patients with FX was 16.9 and that of AFS was 27.7 by Tanaka Binet Intellectual Scale. The most remarkable clinical finding was macroorchidism which was found in 9 out of 11 patients with FX. No macroorchidism was not found in those with AFS. A mean tecticular volume of those with FX was 25.4ml for right and 24.3 for left. No significant correlations were obtained between testicular volumes and individual frequency of FX and also, between individual IQ and FX frequency. In the patients with FX, other clinical features were hyperextensibility of finger joints, high arched palate, articulation errors and hyperreflexia of lower limbs. Relatively striking behavioral feature was withdrawn tendency which was found in 6 patients. No behavioral characteristics were not found in the patients with AFS.

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RADIATION-INDUCED CHROMOSOME DAMAGE IN SPERMATOZOA: COMPARISON BETWEEN HUMAN AND OTHER MAMMALIAN SPECIES. Yujiroh KAMIGUCHI, Hiroyuki TATENO, Masayuki SHIMADA and Kazuya MIKAMO (Dept. of Biol. Sci., Asahikawa Med. Col., Asahikawa)

We studied the effects of X-irradiation on sperm chromosomes of the golden hamster (GH) and the Chinese hamster (CH), and compared these results with our previous data on human spermatozoa. Mature male hamsters were irradiated with 25 to 400 rad of X-rays on their lower abdomen and mated with normal females within 5 days after the exposure. Chromosome slides were prepared at 1st cleavage metaphase, and 1,387 and 342 sperm-derived chromosome complements were analyzed in GH and CH, respectively. The results are as follows: (1) Incidences of spermatozoa with structural chromosome aberrations increased linearly with increasing X-ray dosage both in GH and CH, reaching 48.1 % at 200 rad in GH and 53.5 % at 400 rad in CH. These incidences were much lower than that in human; 3/4 and 2/5. (2) Both breakage-type and exchange-type aberrations showed linear dose-dependent increase in GH as well as in CH. (3) In GH, the incidence of breakage-type aberrations was far higher than that of exchange-type aberrations, as was previously found in human, whereas these two types of aberrations occurred at similar incidences in CH. These results suggest that the CH egg may have higher ability to repair X-ray-induced sperm DNA damage than the GH egg.

A 28

X-RAY INDUCED SCE AND CHROMOSOMAL ABERRATIONS IN THE LYMPHOCYTES FROM THE PATIENTS WITH VON RECKLINGHAUSEN DISEASE.
Hitoshi HOSHINO, Tatsuya TAKESHITA, Chiaki ARIIZUMI, Sumio IIJIMA, Makoto HIGURASHI (Dept. Hlth. Sci., Yamanashi Med. Col., Yamanashi) and Masaya SEGAWA (Segawa neurological clinic for children, Tokyo)

Von Recklinghausen disease, an autosomal dominant disease, is diagnosed with more than 6 Café-au-lait spots and neurofibromas in late childhood or adolescence.

Frequencies of sister chromatid exchanges(SCEs) and chromosomal aberrations (dicentrics and rings) were examined in the cultured lymphocytes from the patients with von Recklinghausen disease and the normal controls after X-ray irradiation at the doses of 250,500 and 750 rads.

The frequencies of SCEs increased with the increasing doses of irradiation both in the patients and the controls.However no apparent differences in SCE frequencies were observed between the patients and the controls.

As well,the frequencies of chromosomal aberrations in the patients were not significantly different from those in the controls.

A 29

GENETIC HETEROGENEITY IN FAMILIAL HYPERCHOLESTEROLEMIA IN JAPANESE.
Kimiko YAMAKAWA, Takaaki OKAFUJI, Yukio IWAMURA, Hisako YANAGI, Yasuko YAMANOUCHI, Kenji YUZAWA, Naoko HATTORI, Shigeru TSUCHIYA*, Koichi KAWAI**, Juichi SATOH** and Hideo HAMAGUCHI (Dept. Hum. Genet., Inst. Basic Med. Sci., *Inst. Comm. Med., **Inst. Clin. Med., Univ Tsukuba, Tsukuba)

Mutations in the gene for LDL receptor give rise to familial hypercholesterolemia (FH). To analyze the genetic heterogeneity due to the differences of allelic mutations in FH in Japanese at the DNA level, we analyzed six FH families by the Southern blot hybridization using the LDL receptor cDNA clone, pLDLR-3 gifted kindly from Dr. Russell, as a probe. In one family, a deletion of 6 kb containing exon 15 or 16 was identified. In another one family, a deletion encompassed exon 16 to 18 was identified. In the third family, a 1.5 kb TaqI fragment was associated with the mutant LDL receptor gene, which has not been detected in unaffected members of the family and 70 unrelated healthy Japanese. In the remaining three families no gross alteration has been identified. Therefore, we determined RFLP haplotypes of the mutant LDL receptor genes in the three families using seven RFLPs in the LDL receptor gene. The RFLP haplotypes differ from one another among the three families. These data indicate that the origin of the six mutant LDL receptor genes are different from one another, suggesting the presence of considerable genetic heterogeneity in FH in Japanese.

A 30

GENETIC POLYMORPHISM OF APOLIPOPROTEIN E AND HYPERLIPIDEMIA IN JAPANESE: IV. APO E4 PHENOTYPE AND HYPERCHOLESTEROLEMIA.
Yasuko YAMANOUCHI, Shigeru TSUCHIYA*, Hisako YANAGI, Ryunosuke MIYAZAKI**, and Hideo HAMAGUCHI (Dep. Hum. Genet., Inst. Basic Med. Sci., *Inst. Comm. Med., Univ. Tsukuba, Tsukuba; **Dep. Med., Kudanzaka Hosp., Tokyo)

Apolipoprotein E (Apo E) phenotypes was determined on 230 apparently healhy unrelated Japanese adults by two-dimensional gel electrophoresis. Gene frequencies were $\varepsilon 3=0.814$, $\varepsilon 4=0.123$, $\varepsilon 2=0.050$, $\varepsilon 5=0.006$, and $\varepsilon 7=0.006$. Among the 230, subjects 108 were males who visited a health care center in Tokyo for thier annual health examinations. Among them, 83 had the apo E 3/3 phenotype, and 25 had the apo E 3/4 phenotype. The mean (±SD) of age was 47.2 ± 6.9 in apo E 3/3 group and 47.0 ± 8.0 in apo E 3/4 group. The mean (±SD) serum cholesterol level in fasting blood was 212.8 ± 41.0 mg/dl in 25 subjects with apo E 3/4 and 196.7 ± 28.5 mg/dl in 83 subjects with apo E 3/3 ($p<0.05$). The frequency of hypercholesterolemia (serum cholesterol levels >250 mg/dl) was 24.0% in 25 subjects with apo E 3/4 and 3.6% in 85 subjects with apo E 3/3 ($p<0.005$). The data suggested that the $\varepsilon 4$ allele might predispose Japanese adults to hypercholesterolemia.

A 31

AN ULTRASONOGRAPHIC DETECTION OF ACHILLES TENDON XANTHOMAS IN FAMILIAL HYPERCHOLESTEROLEMIA. Kenji YUZAWA*, Eriko TOHNO**, Kimiko YAMAKAWA*, Hisako YANAGI*, Takaaki OKAFUJI*, Yasuko YAMANOUCHI*, Naoko HATTORI*, Shigeru TSUCHIYA***, Masayoshi AKISADA**, and Hideo HAMAGUCHI* (*Dept. Hum. Genet., **Dept. Radiol., and ***Dept. Comm. Med., Univ. Tsukuba, Tsukuba)

The diagnosis of familial hypercholesterolemia (FH) is frequently made on clinical ground with detection of tendon xanthoma. In order to clarify whether ultrasonography (US) can be used as a reliable and practical method for detection of Achilles tendon xanthoma in FH, the Achilles tendon thickness in the sagittal section was examined by US in 15 patients with heterozygous FH and 34 normocholsterolemic subjects. The Achilles tendon thickness determined by US was correlated very well with that measured by conventional radiography with a correlation coefficient of 0.99. The mean values and SD of the Achilles tendon thickness determined by US were 4.5±0.5 mm in the normal controls and 11.9±5.1 mm in the patients and the difference was significant ($P < 0.001$). In 13 of 15 patients, US visualized thickened Achilles tendon with convex shape. US could detect Achilles tendons thickened by xanthoma. We conclude that US is a useful aid in the clinical diagnosis of FH.

A 32

A DISEASE WITH REDUCED ELASTIC FIBERS AND AN ABNORMAL ALPHA 2(1) CHAIN OF THE COLLAGEN OF THE SKIN: REPORT OF A MOTHER AND DAUGHTER. Tadashi KAJII, Masato TSUKAHARA, Tsuyako EGUCHI (Dept. Pediatr., Yamaguchi Univ. Sch. Med. Ube), Chidori ASAGAMI (Dept. Dermatl., Yamaguchi Univ. Sch. Med. Ube) and Hiroshi SHINKAI (Dept. Dermatl., Med. Coll. Oita)

A mother and daughter are described with light and electron microscopic, and biochemical abnormalities of the connective tissue of both cutis laxa and the Ehlers-Danlos syndrome. The mother was clinically normal, while her 8-year-old daughter exhibited loose, wrinkled skin and other clinical features of cutis laxa, and also fragility, bruisability and hyperextensibility of the skin and poor healing of the wounds, leaving "cigarette paper" scars, features characteristic of the Ehlers-Danlos syndrome. Light and electron microscopic studies of skin biopsy specimens and cultured skin fibroblasts from both individuals revealed reduced and distorted elastic fibers, a finding usually seen in cutis laxa. Electrophoretic studies of collagen excreted from cultured skin fibroblasts revealed in both individuals an alpha 2(1) chain with a molecular size smaller than usual. The father and elder daughter were normal on clinical, light and electron microscopic and electrophoretic studies.

It was concluded from these findings that the mother and daughter represented a hitherto undescribed disease of the connective tissue with doninant inheritance and variable expressivity.

A 33

A CASE OF HHH SYNDROME: A STUDY ON ORNITHINE METABOLISM IN VIVO USING A STABLE ISOTOPE. Seiichi TSUJINO, Sadayoshi HIGA, Tomokazu SUZUKI Ryuzo MIZUNO, Tsutomu AZUMA, Saburo SAKODA and Susumu KISHIMOTO (3rd. Dept. Int. Med., Osaka Univ. Hosp., Osaka)

A patient with the hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome was reported. The patient, 40-year-old male, was born in Onomichi city, Hiroshima prefecture after an uncomplicated pregnancy and delivery. The parents were unrelated. He has been mentally returded since childhood, and handicapped in walking. At the age of about 13 years, he began to have coma attacks every 2-3 months, which continued for about a day. He was admitted to our hospital in 1986, because of hyperammonemia. On physical examination, pyramidal and cerebellar signs were observed. Biochemical examinations revealed hyperammonemia (344 μg/dl), hyperornithinemia (586.1 nmol/ml) and homocitrullinuria (864 μmol/l). Administration of ornithine HCl (4.8 g/day) reduced the blood ammonia concentrations and prevented the coma attacks. To study the kinetics of ornithine in this condition, stable isotope-labeled arginine (Arg-d_7, 42.5 mg) was administered intravenously into the patient. The concentrations of labeled ornithine (Orn-d_7) in blood and urine was determined by gas chromatography-mass spectrometry. The elimination of Orn-d_7 from plama was slightly delayed in the patient, compared with that of a control subject.

A 34

PROPERTY OF RESIDUAL CATALASE IN THE BLOOD AND CULTURED FIBROBLASTS OF JAPANESE ACATALASEMIA.
Yukinori SATOH, Masana OGATA (Dep. Public Health, Okayama Univ., Okayama)

Hemolysates of normal, heterozygous hypocatalasemic and acatalasemic subjects were separated into three fractions, A,B and C by DEAE cellulose column chromatography, and pI values of A,B and C fractions were determined by isoelectric focusing. The pI value of catalase in the A, B and C fractions increased in the order of normal, hypocatalasemic and acatalasemic mouse bloods. On the other hand, the results obtained from the Japanese acatalasemic blood showed that the pI values of catalase in the A,B and C fractions were similar to those in normal blood. Catalase in Japanese acatalasemic cultured skin fibroblasts was also analyzed by isoelectric focusing. The pI value of catalase in the extract from the cultured skin acatalasemic fibroblasts was similar to that in normal fibroblasts. Catalase C fractions from acatalasemic hemolysate and cultured skin fibroblasts were separated by isoelectric focusing, and transfered to PVDF transfer membrane by electric bloting, and reacted with antibody-antigen complexes on 'Western blots'. Results showed the presence of catalase protein in both specimens.

A 35

NEW THALASSEMIA GENE FOUND IN JAPAN

Yuji NARITOMI, Kunihiro TOKIYAMA, Yutaka CHIFU, Eisuke YOKOTA (1st. Dept. Int. Med. Kyushu Univ., Fukuoka) Hitoshi NAKASHIMA, Takashi IMAMURA (Dept. Hum. Genet. Natl. Inst. Genet., Mishima)

New one base substitution in codon 110 of the human β globin gene was identified in a 55 year old japanese female. Laboratory studies disclosed: Hb 9.1g/dl, RBC 388×10^4, Ht 30%, MCV 77.3 μm^3, MCH 23.5pg, Reticulocytes 38‰. Peripheral blood smear showed many taget cells and fragmented cells. Hb A_2 and Hb F comprised 2.7% and 15.6% of the total hemoglobin. Abnormal hemoglobin was not detected on HPLC. From these results a dignosis of $\delta\beta$ thalassemia was made. No deletion was detected in β globin gene by Sothern blotting. A T→C transition in codon 110 (3rd exon) was identified by the complete sequence of the β globin gene. This transition, has not reported yet, causes the substitution of the leucine residue for proline residue. Because of this substitution at position 110 in the G helix of the β globin chain, both molecular stability of the β globin subunit and $\alpha\beta$ dimer formation, the first step in hemoglobin tetramer formation, will be impeded and the $\delta\beta$ thalassemia phenotype results. The mutation can be idntified upon Msp I digestion. This detection of the mutation on the gene level is of significant advantage for differential diagnostic purposes.

A 36

遺伝子産物の質的異常と量的異常とのオーバーラップ：サラセミックヘモグロビノパチーの分子遺伝学的解析：服巻保幸[1]・小林靖[1]・小松則夫[2]・大庭雄三[3]・宮地隆興[3]・三浦恭定[2]（[1]九大・遺伝情報、[2]自治医大・血液、[3]山口大・臨床検査）：MOLECULAR ANALYSIS OF THALASSEMIC HEMOGLOBINOPATHY. Yasuyuki Fukumaki[1], Yasushi Kobayashi[1], Norio Komatsu[2], Yuzo Ohba[3], Takaoki Miyaji[3] and Yasusada Miura[2] ([1]Research Laboratory for Genetic Information, Fukuoka; [2]Division of Hematology, Jichi Medical School, Tochigi; [3]Department of Clinical Pathology, Yamaguchi University, Ube)

遺伝病を遺伝子産物の質的異常に基づくものと量的異常に基づくものとに区別して考えることは、分子レベルの病因を予想する際有効な場合が多い、しかし中には、両者が同一の病因で生じることがある。βサラセミアはβグロビン鎖の合成障害に基づく疾患であるが、我々はβグロビン鎖の構造変化によりβサラセミアを呈する疾患（サラセミックヘモグロビノパチー）を見い出した。患者は小球性低色素性貧血を呈し、β／αグロビン鎖合成の低下を認めヘテロ接合型のβサラセミアと診断された。患者のβグロビン遺伝子および、網状赤血球を用いて標識アミノ酸存在下で合成したグロビン鎖の解析から次の結果を得た。1）βグロビン遺伝子の一方のalleleに、１１０番アミノ酸LeuからProへの変化を来す一塩基置換を認めた。2）この置換により新たにMspIサイトが出現する。これを利用してSouthern法により患者と同様な貧血を呈する母、姉にもこの変異を検出した。3）逆相ＨＰＬＣによる標識グロビン鎖の解析では、合成時間１５分、２時間いずれにおいても変異グロビン鎖を検出できなかった。１１０番アミノ酸はG-helixに位置しており、Proへの置換によりα-helixが破壊される。従って置換の近傍に位置する α_1-β_1 接触部位が障害を受け、変異グロビン鎖が翻訳後早期に崩壊する為、βサラセミアを呈するものと考えられた。

A 37

HOMOZYGOUS β^0-THALASSEMIA IN A JAPANESE FAMILY. Eisuke YOKOTA, Yutaka CHIFU, Yuji NARITOMI, Kunihiro TOKIYAMA, Yoshiyuki NIHO, (First Dept. Int. Med., Kyushu Univ., Fukuoka) Toshiro HARA (Dept. Pediatrics, Kyushu Univ., Fukuoka) Hitoshi NAKASHIMA, Takashi IMAMURA (Dept. Hum. Genet., Natl. Inst. Genet., Mishima)

A very rare homozygous β^0-thalassemia was found in a Japanese family. The proband, a 6-year-old Japanese girl, was admitted to the Kyushu University Hospital in June 1987. On admission the patient appeared to be pale and slightly icteric. The liver was palpable 4cm below the right costal margin. Hemoglobin was 7.8 g/dl, red blood cells 3.22 million/cmm, MCH 24.2 pg and reticulocytes 9.5%. Peripheral blood smear showed markedly deformed and poorly hemoglobinized red cells with numerous target cells. Bone marrow aspiration disclosed erythroid hyperplasia. HbA was not detected either by HPLC or by electrophoresis on a cellulose acetate sheet. HbF and HbA_2 comprised 98.4% and 1.4% of the total hemoglobins. From these results a diagnosis of homozygous β^0-thalassemia was made. HbA_2 in both parents and younger brother were 6.1%, 5.0% and 5.6%. Therefore all three family members were thought to be heterozygous β-thalassemia. The genomic DNAs from white blood cells of the proband and family members were obtained and structural analysis of β globin gene region is now under investigation.

A 38

Adenosine Deaminase(ADA) Overproduction Associated With Congenital Hemolytic Anemia: Case Report and Molecular Analysis: Hitoshi KANNO, Kenzaburo TANI(Dept.Int.Med.,Inst.Med.Sci.,Univ.Tokyo) Hisaichi FUJII (Dept.Blood Trans.,Med.,Tokyo Women's Med.College) Shiro MIWA(Okinaka Memorial Inst. Med. Res)

We report the fourth case of adenosine deaminase(ADA) overproduction associated with hereditary nonspherocytic hemolytic anemia and the molecular analysis of this anomaly. The proband was a 10 year-old male who had an episode of erythroblastosis fetalis during the perinatal period. The red cell ADA activity was 110-fold increased, but the lymphocyte ADA activity was within the normal range. Western blotting of partially purified ADA from red cells revealed an increased amount of enzyme in the patient's red cells. No gene amplification or gene rearrangement was found by Southern blot analysis, and no increase of ADA mRNA in reticulocyte RNA was detected by dot blot analysis using ADA cDNA. We constructed a patient's genomic library and obtained three clones containing the 5'-promoter region of ADA gene. 2.2kb promoter fragment of these clones was fused to the chloramphenicol acetyl transferase(CAT) gene and transfected to human erythroid cell line of K562, and assayed for CAT activity. One of the clones expressed higher CAT activity than the normal ADA. From these results, 5'-promoter fragment of the ADA gene of the patient was considered to be responsible for the cell specific enhancement of protein synthesis.

A 39

MOLECULAR CLONING AND SEQUENCE ANALYSIS OF MUTANT ALLELES OF ADENYLATE KINASE DEFICIENCY. Shinya MATSUURA, Mie IGARASHI, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med. Ube), Fumio KISHI, Atsushi NAKAZAWA (Dept. Biochem., Yamaguchi Univ. Sch. Med. Ube), Hisaichi FUJII (Dept. Transfusion Service., Tokyo Women's Med. Coll. Tokyo), Shiro MIWA (Okinawa Memorial Institute for Med. Res. Tokyo) and Michiro SAKURAI (Enshu General Hosp. Hamamatsu)

Adenylate kinase (AK1) deficiency is a rare genetic disorder associated with hemolytic anemia. Nine clones containing the AK1 gene were isolated from a Japanese patient using λEMBL4 vector. Nucleotide sequence analysis revealed that they cover both alleles of the patient. One group of clones had a polymorphic base change in the intron 3, while the other had a base change in the exon 6, which brought about an Arg→Trp substitution at the residue 128. The same substitution resulted in a decreased enzyme activity in chicken AK1 as analyzed by the site-directed mutagenesis technique.

A 40

LINKAGE STUDY OF HUNCHINGTON DISEASE TO D4S10(G8 PROBE) IN JAPANESE PEDIGREES. Ikuko KONDO(Dept. of Human Genet., Univ. of Tsukuba, Ibaraki), Ichiro KANAZAWA(Dept. of Neurol., Univ. of Tsukuba, Ibaraki), Joue IKEDA(National Inst. of Agguliculture, Ibaraki), Yuichiro Shizu (Ishizu Hosp., Ibaraki), Rudlph E. Tanzi(Massachusetts General Hosp., Boston, MA, USA) and James F. Gussella(MGH, Boston, MA, USA)

Hunchington disease(HD) is a progressive neurogenerative disorder with autosomal dominant inheritance. HD has been found tightly linked to G8(D4S10), a polymorphic marker(Gusella et al., 1983). The G8 marker detected at least three RFLPs: with Hind III, EcoR I and Bgl I in a Japanese population. Gene frequencies of these polymorphic markers were very similar to those in Caucasian. Four families with HD have been examined for linkage between HD gene and D4S10(G8) in Japanese pedigrees. Three families provided very close linkage(Lod score 2.28 at θ=0.00), but one recombinat could be detected one small family. The maximum lod score for HD against D4S10 was 1.45 at a θ of 0.05, suggesting that there are no evidence of heterogenity of linkage of HD to D4S10 in different human rases, because the maximum lod score was 87.2 at a recombination fraction of 0.03 in males and 0.04 in females in 63 families which were Caucasian and black pedigrees.

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DETECTION OF MISMATCHES IN RNA:DNA DUPLEXES. Keiko HIYAMA, Mieko KODAIRA and Chiyoko SATOH (Dept. Genet., RERF, Hiroshima)

For a study determining nucleotide mutation rates, it is necessary to choose a method which can examine as many base pairs (bp) as possible at one time. The method of "RNase cleavage at mismatches in RNA:DNA duplexes" reported by R. Myers seems to be the most suitable among those available at present. Human DNA segments of 604 bp and 724 bp obtained from cloned normal and thalassemia ß-globin genes, the former being a segment around the capping site and the latter being a segment mainly containing IVS2, were inserted into transcription vectors. Both sense and antisense RNA probes labeled with ^{32}P were made and hybridized with cloned normal and 3 kinds of thalassemia DNAs. Most of the experimental conditions employed were the same as those of Myers but a mixture of RNaseA and RNaseT1 was used to cleave mismatches. Since these DNA segments contain polymorphic base substitutions as well as the deletions or substitutions which cause thalassemia, we could examine 8 out of total of 12 mismatches. Two of them were not cleaved but the mismatches for the same base substitutions in the duplexes made from the opposite combination of strands could be cleaved. Deletions of 1 base (G) and 4 bases (TTCT) were detected in 12 of 12 trials when normal-type RNA probes were employed. The polymorphic substitution of T to C at 666 of IVS2 was detected in DNAs from cell lines established from peripheral B-lymphocytes of healthy individuals.

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Isolation of polymorphic DNA probes containing minisatellite

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A number of hypervariable regions have been discovered in human DNA. They usually consist of tandem repeats of a short sequence that are called minisatellite. Because of the hypervariability a minisatellite could be useful for genetic markers in linkage analysis of human pedigree. In order to isolate polymorphic probes containing minisatellites, we constructed a charomid library. Human DNA was digested with Sau3A completely and fragments longer than three kilobases were isolated, ligated with charomid vector and packaged in vitro. This library, together with a phage library, was screened with three kinds of minisatellite probes designated as myo, core and ins. The sequences comprising 33, 16 and 14 nucleotides, respectively, were synthesized, ligated tandemly and cloned into pUC119. Screening hundred thousands of colonies and plaques, we isolated nine and two polymorphic DNA probes, respectively. One of the probes revealed 14 resolvable alleles among 11 unrelated individuals, and all individuals are heterozygous. The other probes also showed heterozygosity (50 to 90%). The marker loci defined by these probes are expected to be highly informative in linkage studies.

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ORGANIZATION OF HUMAN IMMUNOGLOBULIN HEAVY CHAIN GENES

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The human immunoglobulin heavy chain genes are located on the chromosome 14. The variable region (V_H) genes are classified into four families,namely V_{H-I}, V_{H-II}, V_{H-III} and V_{H-IV}. V_H segments belonging to each family are interspersed among each other in the genome. We have been studying their organization by pulse field gel electrophoresis (PFGE) or isolation of cosmid clones and obtained the following results. 1) Isolated clones were classified into 17 different groups by end labeling and by primer extention method, the longest of them is 130 kb region containing 14 V_H segments. 2) The total length of the V_H locus was estimated to be about 3000 kb. 3) A 300-kb MluI fragment contains V_H, D and J_H segments, indicating thea the distance between V_H and J_H segments is at most 300 kb. 4) Overlapping cosmid clones containing the D_1 D_4, J_H, C and C genes were isolated and the distance between D_3 (most downstream D) and J_H was found to be 22 kb. 5) A novel D segment, located in the region different from the known D region was sequenced and turned out to be potentially functional gene.

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STRUCTURAL AND FUNCTIONAL ANALYSIS OF HLA CLASS II GENES.

Michio YASUNAMI, Akinori KIMURA, Kenji HIRAYAMA, Mitsuru FUKUNAGA, Kazuhiko FUJISAWA and Takehiko SASAZUKI
(Dept. Genet., Med. Inst. Bioreg.,Kyushu Univ.,Fukuoka)

HLA class II genes, DR, DQ and DP genes have been generated by gene duplication events from a common ancestral gene and there is structural diversification among these genes which may account for the functional divergency. In comparison with DR antigen, DQ antigen has a weak stimulatory effect on allogeneic MLR and is less contributive to antigen presentation to specific T lymphocyte proliferation. To investigate separately the functions of DR and DQ molecules, we established HLA class II gene transfectants. DRα,DQα and DQβ genes isolated from Dw12 as well as DQα and DQβ genes from Dw15 and a DRβ cDNA from Dw12 under SV40 promoter were introduced into murine L cells and expression of class II molecules was certicified by flowcytometric analysis. Both DR and DQ transfectants of Dw12 stimulated the secondary MLR primed with cells from a Dw12 positive individual, and the responses were blocked by relevant monoclonal antibodies. While the DR transfectant stimulated primary proliferative response, the DQ transfectant could not. The result indicated that DQ molecule have potentially a stimulatory effect with alternate kinetics in primary response.

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HLA CLASS II TRANSGENIC MOUSE Kenji HIRAYAMA, Tomohisa IWANAGA, Akinori KIMURA, Michio YASUNAMI and Takehiko SASAZUKI. Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka.

We have already reported that HLA-class II molecules played important roles in the regulation of immune response to natural antigens, such as streptococcal antigen, cedar pollen antigen or schistosomal antigen in humans. To investigate the immunological function of HLA-classII genes in ontogeny, cloned HLA-DR α or HLA-DQ α, β genes from homozygote for HLA-Dw12 were introduced into fertilized egg from C57BL/6 mouse to establish an HLA-classII transgenic mouse. Northern blot analysis showed that mRNA of HLA-DR was expressed in spleen and thymus but not in liver, thyroid or kidney. The expression of DQw1 molecule in both spleen and thymus of HLA-DQ α, β transgenic mouse was observed by tissue staining with anti HLA-DQw1 monoclonal antibody, HU-11. The expression of these transgenes was suggested to be controlled in a tissue specific manner. The immunological function of these transgenes is now under investigation.

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IDENTIFICATION OF NUCLEAR FACTORS WHICH BIND TO CONSERVED NUCLEOTIDE SEQUENCES AMONG VARIOUS HLA CLASS II PROMOTERS. Akinori KIMURA and Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

To investigate the mechanism of tissue specific regulation of HLA class II gene expression, we have employed gelshift assay, DNase I footprinting and South-Western blotting to analyze nuclear extracts from various cell lines and identified at least four nuclear proteins which bind specifically to conserved nucleotide sequences among HLA class II promoters. A factor that recognize Y box sequence was ubiquitous in all cell types tested. Although this factor was similar to CTF, composition of the factor was different from cell type to cell type and its binding affirnity with various class II promoters was in negative correlation with their potential strength. Octamer binding factor and X box binding factor seemed to be abundant in B cells expressing class II genes whereas W box binding factor that may confer to inducibility of HLA class II gene expression by IFN γ was more abundant in fibroblasts negative of class II gene expression. These results indicate that the HLA class II genes are regulated in tissue specific and gene specific manners by tissue specific positive and negative transacting factors.

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Immune suppression gene on HLA-Bw54-DR4-DRw53 haplotype controls nonresponsiveness in humans to HBsAg via CD8+ suppressor T cells.
Hiroshi WATANABE, Kenji HIRAYAMA, Takehiko SASAZUKI (Dept. Genet., Kyushyu Univ., Fukuoka)

We immunized 85 healthy volunteers with hepatitis B(HB) vaccine, and even after the third immunization, 22.4% of the vaccinees show no evidence of anti-HBs production, in vivo and in vitro. And this nonresponders showed a strong association with HLA-Bw54, DR4, DRw53, and HLA-Bw54-DR4-DRw53 haplotype. The strong association and high frequency of HLA-DRw53 in the nonresponders, made feasible application of the method of Thomson & Bodmer to test the mode of inheritance of nonresponsiveness to HBsAg. To use HLA-DRw53, genetic analysis revealed that the nonresponsiveness to HBsAg is an HLA-linked dominant trait rather than recessive trait. Then, the nonresponsiveness to HBsAg is controlled by the presense of certain cells and/or molecules than the absence of same. PBL from nonresponders posessed a CD8 positive suppressor T cells which abolished the anti-HBs production, in vitro, in an antigen specific manner. Thus, the nonresponsiveness to HBsAg is controlled by an HLA-linked immune suppression gene for HBsAg (Is-HBsAg).

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PSORIASIS AND HLA.
Masahiko MUTO, Hideto KIMURA, Yoshio NAKAMIZO (Dept. Dermatol., Kyushu Univ., Oita), Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka)

Psoriasis is considered a genetic disease. However its pathogenesis has not been clear. In order to clarify genetic involvements for the development of the disease, we performed family study and population survey. Family analysis revealed that the susceptibility to psoriasis vulgaris (PV) might be a multifactorial trait with more than 80 percent heritability. From a population study, with HLA marker, using 44 patients with PV and 13 with psoriatic arthropathy (PA), PV showed associations with HLA-Cw6 and Cw7 (primary association was with HLA-Cw6). In addition, PA showed associations with HLA-A2, B27, Bw46, Cw6, and DRw8 (primary association was with HLA-B27). The present results in PV were similar to those of the preveous reports. It is a new finding that HLA-A2, Bw46, and DRw8 had relative strong associations with PA. Furthermore, it is very interesting that PA did not show an association with HLA-DR4, which is strongly associated with rheumatoid arthritis. This finding suggests that PA is a genetic disorder different from PV and rheumatoid arthritis.

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STRUCTURE OF THE HUMAN ORNITHINE TRANSCARBAMYLASE GENE.
Akira HATA*,**, Teruhisa TSUZUKI*, Kazunori SHIMADA*, Ichiro MATSUDA**(*Dept. Biochem.,**Dept. Pediatr., Kumamoto Univ., Kumamoto)

Ornithine transcarbamylase (OTC), the second enzyme of the urea cycle, is a nuclear DNA coded mitochondrial protein. OTC deficiency is the most common inborn error of the urea cycle and shows an X-linked inheritance. We considered that molecular cloning and characterization of the normal human OTC gene are prerequisites for elucidating the regulatory mechanism of OTC gene expression, as well as for developing a DNA-related diagnosis of OTC deficiency. Complementary DNA clones corresponding to the human OTC mRNA and genomic DNA clones have been isolated and analyzed. The OTC gene is about 73 kilobase pairs (kb) long and contains ten exons interrupted by nine introns of highly variable sizes. The smallest intron is 80 base pairs and largest, 21.7 kb. The 5'- and 3'-flanking regions, entire exons and all the exon/intron boundaries were sequenced. The nucleotide and deduced amino acid sequences of isolated OTC cDNAs as well as the corresponding regions of the genomic DNA were compared with those of human OTC cDNA (Horwich, A.L. et al. 1984 Science 224, 1068-1074). We found 21 nucleotide substitutions among these sequences, six of which were related to amino acid changes.

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STRUCTURE OF THE GENE FOR HUMAN LIVER ARGINASE AND RFLPs
Yougo HARAGUCHI, Masaki TAKIGUCHI, Masataka MORI (Inst. Med. Genet., Kumamoto Univ., Kumamoto) and Ichiro MATSUDA (Dept. Pediatr., Kumamoto Univ., Kumamoto)

Liver-type arginase (EC 3.5.3.1) is localized in the cytosol of hepatocytes and catalyzes the last step of the urea synthetic pathway. Inherited deficiency of the enzyme results in argininemia, an autosomal recessive disorder accompanied by hyperammonemia. The gene for the human enzyme was cloned and the structure was determined. This gene is 12 kilobases long and is split into 8 exons. All of the splice donor and acceptor sites conform to the GT/AG rule. The transcription start site was determined by primer extension and S1-mapping. A "TATA box"- and a "CAAT box"-like sequences are located 28 and 72 bases upstream from the start site, respectively. In the 5' end region, sequences resembling the glucocorticoid receptor binding sites and enhancer core sequences, and several sets of direct repeat and inverted repeat are present. The exon-intron organization of the human arginase gene is very similar with that of the rat gene. There are several highly conserved segments in the 5'- and 3'-flanking regions between the human and rat genes. Two restriction fragment length polymorphisms were identified at the arginase gene locus, using restriction endonucleases PvuII and HincII.

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TRANSFECTION OF HUMAN MALE CELL WITH PLASMID WHICH CONTAINS Y-CHROMOSOME SPECIFIC REPEATED DNA. Yutaka NAKAHORI, Masao YAMADA, Yasuo NAKAGOME (National Children's Medical Research Center)

It has been reported that an exogenous DNA transfected to the cultured mammalian cell can be integrated to the chromosome DNA according to the sequence homology between them. 3.4Kb Y-chromosome specific repeated DNA is reiterated 3000 times on the Y-chromosome long arm. We made plasmid which contains this repeated DNA and the 3'phosphotransferase gene that confers Neomycin resistance. Using calcium phosphate method, we transfected cultured human male cell with the plasmid. After the selection with the medium which contains 400ug/ml Neomycin, the Neomycin resistant cells were cloned and DNA was extracted from them. We have analysed the recombination point in plasmids and chromosomes by Southern blot hybridization.

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PEPSINOGEN GENE COMPLEX (PGA) WAS ASSIGNED TO HUMAN CHROMOSOME 11q13 BY *in situ* HYBRIDIZATION. Hiroshi NAKAI, Kiyoshi HASEGAWA, Yoshitsugu YAMAMOTO, Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai), M.G. BYERS, T.B. SHOWS (Dept. Human Genet., Roswell Park Memorial Institute, USA) and R.T. TAGGART (Dept. Med., UCLA, USA)

Human Pepsinogen (PGA3, PGA4 and PGA5) were assigned to chromosome region 11q13 by *in situ* hybridization.

Pepsinogen is major acid proteinase precurcer secreted by the gastric mucosa. The fractions Pg3, Pg4, Pg5 are products of PGA3, PGA4, and PGA5 gene respectively. In previous mapping study using human-mouse hybrid cells, the gene locus of pepsinogen was indicated on chromosome 11p11-q13 (Taggart et al. 1986). We used a 705bp cDNA probe specific for exons 3 through 8 of the nine-exon-long PGA gene which was isolated from human gastric mucosa cDNA library. Method were followed to Zabel et al. (1983). A total of 158 metaphases were examined and 10.1% of these revealed silver grains at 11q13. Chromosome 11 had 12.6% of total 356 grains and 5% of the grains were concentrated over the 11q13 region. This was a significantly high peak. Then 11q13 was decided as the locus of human pepsinogen gene complex.

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AN APPROACH TO MOLECULAR BASIS OF MORPHOGENESIS IN TRANSGENIC MICE
Tomohisa IWANAGA, Shoji WAKASUGI, Takeaki INOMOTO, Masahiro UEHIRA, Kimi ARAKI, Jun-ichi MIYAZAKI, Ken-ichi YAMAMURA (Inst. Med. Genet., Kumamoto Univ. Med. Sch., Kumamoto), Shuichiro MAEDA & Kazunori SHIMADA (1st Dept. Biochem., Kumamoto Univ. Med. Sch., Kumamoto)

It has been difficult to characterize the molecular events that guide morphological pattern formation during embryonic developement. One promising approach towards molecular basis of morphogenesis is to analyze transgenic mice which have a dysmorphic mutation.

Recently, we have created transgenic mice harboring a variant human transthyretin (v-hTTR) gene in an attempt to make animal models of familial amyloidotic polyneuropathy. Out of 9 transgenic mice, we discovered one which has facial malformation. This mutant phenotype is inherited in an autosomal dominant manner, and co-segregates with the introduced gene. This mutation may have been caused either by insertional mutagenesis or aberrant expression of the v-hTTR gene itself. To test the former possibility, we have cloned several DNA fragments flanking the integrated v-hTTR gene and now are studying if these fragments are parts of a functional gene. Also, we have examined the pattern of v-hTTR gene expression during gestation to test the latter possibility.

A 54

トランスジェニックマウスでのヒト血清アミロイドP成分遺伝子の発現とそのアミロイドーシスにおける役割の解析．井本岳秋1.若杉正司1.岩永知久1.上平昌弘1.荒木喜美1.宮崎純一1.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 AMYLOID P-COMPONENT GENE IN TRANSGENIC MICE.
Takeaki INOMOTO 1, Shoji WAKASUGI 1, Tomohisa IWANAGA 1, Masahiro UEHIRA 1, Kimi ARAKI 1, Jun-ichi MIYAZAKI 1, Shuichiro 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)

種々のアミロイドーシスで沈着する異なるアミロイドの共通成分、血清アミロイドP成分(SAP) がアミロイドの沈着にどのように関与するかを調べるために、5`上流0.7 kb及び3`下流1.2 kbを含むヒト全SAP 遺伝子を組み込んだトランスジェニックマウスを作成した。得られた8 匹のマウスについては、組み込まれたヒトSAP 遺伝子のコピー数と肝中のヒトSAP mRNA量とに明らかな相関が認められた。マウス血中のヒトSAP 量と肝中のSAP mRNA量とにも明らかな相関が認められた。マウスの種々の組織中のヒトSAP mRNA量の存在を調べたところ、肝にのみヒトSAP mRNAを見出した。さらにマウス肝中のヒトSAP mRNAはマウス腹空内に細菌リポ多糖体を投与後24～48時間で2-3 倍に増加した。

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DEVELOPMENT OF A PARTIAL-MODEL MOUSE OF DOWN SYNDROME. Futoshi SHIBATA, Nobuyuki KUROSAWA, Tohru YOKOI, Kazuko HAYASHI and Zen-ichi OGITA (Dept. Pathogenic Biochemistry, Res. Inst. for Oriental Medicines, Toyama Med.& Pharmaceut. Univ. Toyama)

As mouse chromosome 16 has a homologous region of human chromosome 21, mouse trisomy 16 has been developed as an animal model of human trisomy 21 (Down syndrome). However, the trisomic fetuses die before birth. Therefore, we attempted to develop a partial-model mouse of Down syndrome, using transgenic mouse carrying an extra copy of one or a few genes located on the distal part of human chromosome 21 (q22.1-q ter) which is the consensus region for Down syndrome phenotype. Human superoxide dismutase-1 (SOD-1) gene with 5' flanking sequence of mouse metallothionein gene was microinjected into fertilized mouse eggs and two transgenic mice were obtained. Expression of transgene is now being analyzed. Mouse SOD-1 gene is now being cloned. Mouse cDNA library was screened using human SOD-1 cDNA (kindly provided by Dr. Y. Groner) as a probe and cDNA clones containing sequences of mouse SOD-1 gene were isolated. Mouse genomic library in lambda EMBL3 was screened with the cloned SOD-1 cDNA and two recombinant phages have been isolated, so far. We wish to isolate a clone containing the whole mouse SOD-1 gene and to produce the transgenic mouse with extra copy of this gene.

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A TRANSGENIC MOUSE MODEL OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY. Shoji WAKASUGI, Takeaki INOMOTO, Masahiro UEHIRA, Tomohisa IWANAGA, Kimi ARAKI, Jun-ichi MIYAZAKI, Ken-ichi YAMAMURA (Inst. Med. Genet., Kumamoto Univ. Med. Sch., Kumamoto), Shigehiro YI, Makoto NAITO, Kiyoshi TAKAHASHI (2nd Dept. Pathol., Kumamoto Univ. Med. Sch., Kumamoto), Shuichiro MAEDA and Kazunori SHIMADA (1st Dept. Biochem., Kumamoto Univ. Med. Sch., Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disorder, characterized by the extracellular deposition of fibrillar amyloid protein and by prominent peripheral nerve involvement. This deposition is mainly composed of transthyretin (TTR) with a substitution of methionine for valine at position 30 in the FAP type I patients. To elucidate other factor(s) than the single amino acid substitution of TTR and to examine the pathological process of amyloid deposition, we have produced transgenic mice by microinjectiong the cloned human mutant TTR gene into fertilized eggs of C57BL/6 mice. Amyloid deposition was observed mainly in the submucosa of the intestine of the transgenic mice at the age of 6 months and more remarkably at the age of 12 months, when about 10 % of the renal glomeruli were also shown to have amyloid deposition. These amyloid substances were stained with anti-human TTR antisera. This result demonstrated that the variant TTR was deposited as amyloid fibrils in these mice.

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核内遺伝子と細胞質遺伝子によるキイロショウジョウバエ寿命の決定。米村 勇・大橋正明・太田正穂・福島弘文・支倉逸人（信州大・医・法医）. Life span determined by nuclear genes and plasmagenes in D. melanogaster. Isamu YONEMURA, Masaaki OHASHI, Masao OTA and Hayato HASEKURA (Dept. Legal Med.,Shinshu Univ.School of Med.,Matsumoto)

寿命・老化は生物学に残された最大の課題と言われている。従来、寿命はポリジーンに支配されているとする説が大勢を占めており、主働遺伝子説を唱える研究者もいるが実験的な裏付けに乏しく殆ど推論の域を出ていなかった。しかし、我々は単純な寿命を示す高度純系キイロショウジョウバエの交配実験により寿命は少数の遺伝子により明確に決定されていることを見いだした。即ち細胞質寿命遺伝子により寿命の大枠が決定され、常染色体性寿命遺伝子により中枠が決定され、最後に性染色体性寿命遺伝子により細かく決定されていることが明らかになった。これらの遺伝子をJm（寿命Ju-myoの意）と名付けた。生命そのものは殆ど無数と言えるほどの多くの遺伝子に依って維持されているが、それら生命維持遺伝子群の活動期間は少数のJm遺伝子の組合せに依って決定されていると考えられる。これにより、少数のJm遺伝子ないしはその産物を操作することにより寿命の人工的操作が可能である事が示されたと言えよう。ヒト寿命に就いてもショウジョウバエと基本的には同様な遺伝的仕組みがある事が示唆された。

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A CASE OF CUTIS LAXA SYNDROME WITH MUSCLE ATROPHY.
Yukihisa MATSUDA, Shigeyuki NAKAMURA, Tshiani KASHAMA, Akihiko KODAMA, Koji SAMESHIMA (Dept. Pediatr., Kagoshima Univ., Kagoshima) and Masanori NAKAGAWA, Mitsuhiro OSAME (3rd Dept. Intern., Kagoshima Univ., Kagoshima)

Cutis laxa syndrome is a rare connective tissue disorders of unknown etiology. It is characterized by reduction in the amount and the size of elastic fibers. The patient was a male infant, born at 39 weeks after an uneventful pregnancy without consanquinity. His mother's first and second pregnancies terminated in spontaneous abortions. He was noted to have an old-appearing face and loose non-hyperelastic skin at birth. His first admission was at the age of 11 months. Then we could diagnose him as cutis laxa syndrome by histological study from the skin biopsy. The serum CPK was normal (50 mu/dl). Till 1 year and 10 months old, he could walk by himself. But he gradually lost the muscular strength of the lower extremity, and showed his Gower's sign on standing. The serum CPK was 230 mu/dl. Therefore we performed the muscle biopsy of the right upper arm. It showed histologically the vacuolar change in the muscle tissues. There were mild to moderate increase of acid phosphatase activity in amount of fibers, and there were mild increases of muscle fibers glycogen content mostly myofibrils.

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Alport 症候群の1家系．小林義治[1]・松井　晶[2]・関はるみ[3]・鈴木　豊[4]・松田健史[5]([1]伊勢崎市民病院眼科・[2]同小児科・[3]同耳鼻科・[4]同病理・[5]富山医科薬科大学第1解剖)．A FAMILY OF ALPORT'S SYNDROME. Yoshiharu KOBAYASHI[1], Akira MATSUI[2], Harumi SEKI[3], Yutaka SUZUKI[4], and Takeshi MATSUDA[5]([1]Dept.Ophthalmol.,[2]Dept.Pediat.,[3]Dept.Otolaryngol.,[4]Dept.Pathol.,Isesaki Municipal Hosp.,Isesaki ; [5]Dept.Anatomy, Toyama Med.& Pharmaceut.Univ.,Toyama)

発端者は12歳、男子。2歳7か月時、感冒に罹患後、肉眼的血尿と蛋白尿[1+]にて発症。3歳10か月、第1回腎生検。光顕所見では散在性に胎児性未熟糸球体が見られた。電顕所見では糸球体基底膜（ＧＢＭ）のごく一部に層状分裂像を観察。蛍光抗体法では主としてメサンギウム領域にＩｇＭの沈着を証明。当時、難聴・眼所見はなかった。蛋白尿は6歳頃より徐々に増加し、10歳頃から[4+]が持続。この頃感音性難聴が出現。10歳10か月時，第2回腎生検。光顕所見では間質に泡沫細胞が見られた他に著変なし。電顕所見ではＧＢＭに広範な層状分裂像を認めた。11歳時、眼底検査で黄斑部周囲に融合傾向のある顆粒状白斑を確認したことを加わえ、9年間の臨床成績から Alport症候群と診断した。発端者の母親 33歳は、13歳頃から腎臓病。顕微鏡的血尿と蛋白尿[2+～3+]が持続するも腎機能は正常。眼科・耳鼻科的に異常なし。腎所見は発端者のそれに類似する。母方の祖父・叔母に顕微鏡的血尿あり。母方の曾祖父は40歳以前に腎臓病で死亡。父親・同胞2人に尿異常なし。以上の如く先の第25回本学会で報告した家族性腎症の2家系のうち1家系について Alport 症候群であることが確認されたのでここに報告した。

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BORJESON-FORSSMAN-LEHMANN SYNDROME: FAMILIAL OCCURRENCE OF DAUGHTER AND HER MOTHER. Kiyoshi IMAIZUMI,YoshikazuKUROKI(Dept.Genet.,Kanagawa Child. Med. Cent.,KANAGAWA), Toshio MIURA(Dept.Pediatr. Yamaguchi Cent. Hosp.,Yamaguchi), Tadashi KAJII(Dept. Pediatr.,Yamaguchi Univ., Yamaguchi)

A 14-year-old girl had typical clinical features of the Börjeson-Forssman-Lehmann syndrome including a mental retardation, short stature, a coarse face with a microcephaly, prominent supraorbital ridges, deep set-eyes with a left divergent strabismus, ptosis of bilateral eyelids, a deep nostrils, soft puffy cheeks, bilateral large ears and small hands with tapering fingers. She showed no secondary sexual characteristics. In previous reports, there were five papers with familial occurrence, so we analysed here these pedigrees including our cases. Results were as followes: 1) The clinical features among the affected females were more moderate than those of males and were highly variable. 2) The syndrome were transmitted vertically through two or three successive generations. 3) Male-to-male transmission were noted only once. 4) The mutant genes were almost transmitted through females. 5) The sex ratio of affected individuals were significantly different from one. 6) The segregation ratio of the families were 0.428±0.006 These situations infact proposed that the inheritance of the BFLS was a X-linked semi-dominant or autosomal dominant with sex influenced mode.

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下垂体性小人症と性腺機能低下，精神遅滞を伴う伴性劣性魚鱗症の新亜型。国見雅子・泉 達郎・斎藤加代子・福山幸夫（東女医大・小児）A NEW SUBTYPE OF X-LINKED ICHTHYOSIS WITH PITURITARY DRAWFISM, HYPOGONADISM AND MENTAL RETARDATION.
Masako KUNIMI, Tatsuro IZUMI, Kayoko SAITO and Yukio FUKUYAMA
(Dept. Pediatr., Tokyo Wom. Med. Coll., Tokyo)

遺伝性角化異常症である魚鱗癬の中で，伴性劣性魚鱗症はSteroid sulfatase 欠損が原因であり，この欠損は患児の白血球，皮膚線維芽細胞で証明できるが保因者である母親においては本酵素の活性値のみの判定は困難といわれている。今回我々は先天性魚鱗症を認める１４歳男児で，Steroid sulfatase 活性を，４Methylumberiferane-Sulfateを基質としてarylsulfatase C 活性で検討しその欠損を証明し，X-linked ichthyosisと診断したが，母親，父親の酵素活性は白血球でそれぞれ，77.0, 81.5 (68.5±17.8)で，この酵素の測定のみでは保因者の診断はできなかった。又患児においては更に下垂体性小人症，停留睾丸，LH-RH 負荷に対するLHとFSH の反応遅延等の性腺機能低下，精神遅滞，左の聾等の合併を認め，又皮膚組織所見でも尋常性魚鱗症の所見を示し，X-linked ichthyosis とは異っており非典型であったので，本症例の特異性や Rud症候群等の既知の症候群との異同について報告した。

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GENETIC AND BIOCHEMICAL STUDIES OF PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY.
Kenichi HORINOUCHI, Munenori IWASE, Akira AKATSUKA, Takeshi SAKIYAMA and Teruo KITAGAWA (Dept. Pediat., Nihon Univ. School of Med., Tokyo)

Purine nucleoside phosphorylase (PNP) deficiency is characterized by severe combined immunodeficiency. The first case, in Japan, was a 7-year-old girl, who had developed recurrent respiratory infections since 3 years old and died from varicella infection. She had significantly low levels of serum uric acid and reduced T-lymphocytes function. The PNP activities in patient's and parents' erythrocytes was only 4.8% and about 50% of normal control respectively. The residual PNP in liver was immunologically cross reactive to the antibody which was determined by Ochtalony and Western blotting. The physico-chemical characteristics of PNP activities from the patient's liver showed no remarkable difference when compared to that of the controls except for the slightly different optimal pH. The mRNA was extracted from the patient's as well as control livers and Northern blotting method was used. The result showed that the patient had the same molecular RNA size and the intact volume of mRNA. These strongly suggests that the patient has point mutation in PNP-DNA.

B 1

EXPRESSION OF Fra(X)(q27.3) ASSOCIATED WITH FRAGILE X SYNDROME.
Tada-aki HORI, Ei-ichi TAKAHASHI, Hideo TSUJI, Satsuki TSUJI (Div. Genet., Natl. Inst. Radiol. Sci., Chiba) and Motoi MURATA (Div. Epidemiol., Chiba Cancer Center, Chiba)

Expression of fra(X)(q27.3) induced by low and high thymidylate stress was studied in thymidine-prototrophic and -auxotrophic human - mouse somatic cell hybrids. Low thymidylate stress achieved by 5-fluoro-2'-deoxyuridine treatment and by limiting the exogenous supply of thymidine induced fragile X expression. High thymidylate stress produced by supplying excess amounts of thymidine was also found to be effective in inducing the fragile X expression even in a hybrid clone which retained a fragile X chromosome as the only human chromosome, and an addition of deoxycytidine completely abolished the effect. Since the elevated levels of dTTP result in a relative deficiency of dCTP by its feedback inhibition of ribonucleotide reductase, these results imply that deficiency of dTTP and dCTP may be the primary biochemical events leading to fragile X expression. Based on these results, we suggest that the expression is an intrinsic property of the fragile site which might be originated from chromosomal mutation, such as duplication or deletion, at pyrimidine-rich DNA sequences in Xq27.3 region of the human X chromosome.

B 2

BrdU DEPENDENCY AND LATERAL ASYMMETRY IN A HERITABLE FRAGILE SITE, fra (10)(q25).
Tatsuro IKEUCHI, Kohtaro YAMAMOTO, Mitsuaki YOSHIDA (Dept. Cytogenet., Tokyo Med. Dent. Univ.), Ei-ichi TAKAHASHI, Tada-aki HORI (Natl. Inst. Radiol. Sci.) and Motoi MURATA (Chiba Cancer Center)

A total of 14 lymphoblastoid cell lines (LCL) were established by EB virus-mediated transformation from heritable fragile site (FS) carriers, who were detected in a general population and in patients with hematopoietic diseases. Expression rates of FS in these LCL were in general very low, but a cell line 'B-3' derived from a fra(10)(q25) carrier expressed the FS with high frequencies (40-60%) after exposure to BrdU (7-10 μg/ml) for 24 hrs. The fra(10) expression was characterized by a predominance of chromatid gaps or breaks rather than of isochromatid types as seen in other kinds of FS. The lateral asymmetry in expression of the FS was presumed, as known in mouse and human heterochromatin, to reflect an unequal distribution of thymine between the 2 strands of DNA duplex in the fra(10) site. This was supported by the following findings: 1) The fra(10) expression was dependent on the incorporation of BrdU into the FS DNA. 2) In the 1st mitotic cells after the BrdU addition, gaps and breaks were mostly of chromatid type, while in the 2nd mitoses the lesions of both chromatid and isochromatid types were almost equally observed. 3) In the 2nd mitoses, the induced FS of chromatid type was invariably associated with the lightly stained chromatid.

B 3

STUDY ON PARENTAL ORIGIN OF EXTRACHROMOSOME IN 4 CASES OF TRISOMY 18 USING RFLP. Hidefumi TONOKI, *Tatsuro KONDOH, Tsutomu KAMEI, Junichi HAMABE, Shigeto SUGINO, Sei OKIMOTO, Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., *Dept. Pediat., Nagasaki Univ., Nagasaki)

There has been only a study on the origin of trisomy 18, because of no heteromorphism on chromosome 18. We attempted to trace the parental origin of an extrachromosome in trisomy 18 using RFLP of human prealbumin gene (PA), of which locus has been assigned to 18p11.1-q12.3. DNAs of 4 standard 18-trisomics and of their parents were digested by MspI and the Southern blots were hybridized to PA-cDNA. Normal controls gave five fragments, of which 1.7kb/0.95kb fragments were polymorphic. Densitometric analysis revealed that Patient 1 had two copies of the 1.7kb and one copy of the 0.95kb, while her father and mother were homozygotes for the 0.95kb and the 1.7kb, respectively. Thus, it is concluded that extrachromosome 18 of this patient was originated in nondisjunction during the maternal meiosis. Likewise, the maternal meiotic errors were identified for the origin of additional #18 in the remaining 3 patients. When our data are combined with the paternal origin in a reported case, nondisjunctions at the maternal and at the paternal meiosis occurred in trisomy 18 at the ratio of 4:1, which is compared to those in trisomies 13 and 21, since all these trisomies are associated with an advanced maternal age.

B 4

CYTOGENETIC CHARACTERISTICS OF THE CULTURED LYMPHOCYTES FROM THE ADULT PATIENTS WITH DOWN SYNDROME. Tatsuya TAKESHITA, Hitoshi HOSHINO, Chiaki ARIIZUMI, Sumio IIJIMA, and Makoto HIGURASHI (Dept. of Health Sci., Yamanashi Medical College, Yamanashi)

Frequencies of dicentrics plus rings per cell after X-ray irradiation in the whole blood at the doses of 250, 500, and 750 rads were about 1.5 times as high in the adult patients with Down syndrome in their 20's (0.33 ± 0.02, 1.01 ± 0.08, 1.39 ± 0.19, respectively) as in the age-matched normal controls (0.32 ± 0.04, 0.61 ± 0.10, 0.94 ± 0.10, respectively). These results were mostly consistent with those in the younger patients. As to the mitogenic responses to PHA, the adult patients (both in their 20's and 40's) showed similar cell cycle kinetics compared to the age-matched normal controls. Mitotic indicies were slightly higher in the adult patients in their 40's compared to the controls of similar ages. No such differences were observed between the patients and controls in their 20's.

B 5

INHERITANCE OF RECIPROCAL TRANSLOCATION CHROMOSOMES IN MAN (3).
Hidetsune OISHI (Dept. Genet., Inst. Develop. Res., Aichi Prefect. Colony, Kasugai), Kaoru SUZUMORI (Dept. Obs. Gynec., Nagoya City Univ., Nagoya), Ken HAYASHI (Dept. Obs. Gynec., Kyoto Univ., Kyoto) and Tsutomu YAMANAKA (Cent. Hosp., Aichi Prefect. Colony, Kasugai)

The frequencies of autosomal rearrangements with reciprocal translocation were estimated from our records and published reports with data of prenatal diagnoses. In 62 families examined for reciprocal translocation of chromosomes, male and female probands were 34 and 28, respectively, while total numbers of balanced carriers with apparently normal phenotype found in these families were 115 males and 160 females. By the pedigree analyses 19 males and 28 females with the same conditions of chromosomal rearrangements for two or more generations were ascertained as the initial balanced carriers. In addition, 144 parents with balanced translocation have had 96 sons and 132 daughters with the same conditions. Following prenatal diagnoses by amniocenteses or chorionic villus sampling, 14 fathers with balanced carriers were found to have 6 sons and 8 daughters with the same chromosomal rearrangements whereas 11 sons and 18 daughters of 28 mothers were under the same situation. The reason for excess existences of these females with balanced translocation is still obscure.

B 6

Sister Chromatid Exchanges in the Senile Dimentia of Alzheimer Type Patients and in the Older Persons.
Kanehisa MORIMOTO, Naoaki NIINO (Dept. of Public Health, Univ. of Tokyo, Tokyo), Kumiko TAKADAYA-IIJIMA, Munehiro HIRAYAMA (Dept. of Maternity & Child Health, Univ. of Tokyo, Tokyo)

The frequencies of baseline and mutagen (mitomycin-C;MMC, 4-nitroquinoline-1-oxide;4NQO)-induced sister chromatid exchanges (SCEs) were examined in the 5 senile dimentia of Alzheimer type (SDAT) patients, the malti-infarct dimentia (MID) patients and 14 healthy elderly subjects. The mean-ages of SDAT, MID and healthy subjects were 84yr, 82yr and 88yr, respectively. There were no significant differences in both baseline and mutagen (MMC, 4NQO)-induced SCE frequencies among these three groups. The pooled data on these subjects (aged 70-98 yrs) were used to examine the effects of aging on SCEs. The baseline SCEs were decreased with aging: $Y = -0.15X + 23.45$ ($r = -0.37$, P 0.05) (X; age in yr: Y; SCEs per cell), however induced SCEs showed no difference.

B 7

DEFECT OF EXCISION REPAIR OF ACTIVE GENES IN COCKAYNE SYNDROME CELLS. Masaru YAMAIZUMI, Tsuyoshi UCHIDA (Institute for Molecular and Cellular Biology, Osaka Univ., Osaka)

Cockayne syndrome(CS) is a rare autosomal recessive disease characterized by clinical manifestations such as photosensitivity, optic atrophy, mental retardation and premature aging. Like xeroderma pigmentosum(XP) cells, cells from patients with the Cockayne syndrome show high sensitivity to ultraviolet(UV) light. However, on examination of their total DNA after UV irradiation the level of unscheduled DNA synthesis(UDS) and the disapperance of pyrimidine dimers were found to be normal. Two characteristics of Cockayne syndrome cells are low recoveries of RNA and DNA syntheses after UV irradiation. Based on these phenotypes, 3 genetic complementation groups have been identified. The primary defect in the Cockayne syndrome is unknown, but from these findings some step after incision in excision repair is thought to be defective. In this study we found that microinjection of T4 endonuclease V restored RNA synthesis in Cockayne syndrome cells of all complementation groups.

B 8

PARTIAL PURIFICATION AND CHARACTERIZATION OF THE FACTOR THAT RESTORES THE DEFECT OF XERODERMA PIGMENTOSUM GROUP A CELLS. TATSUO SUGANO, MASARU YAMAIZUMI, HIROSHI ASAHINA, TSUYOSHI UCCHIDA (Institute for Molecular and Cellular Biology,Osaka University)

Xeroderma Pigmentosum(XP) is an autosomal recessive disease in which patients show a high incidence of skin cancers often accompanied with neurological simptoms.Cultured fibroblasts from XP patients are remarkably sensitive to ultraviolet light. Defect in excision repair at the initial step(incision) has been sugested in these cells. There are at least 9 genetic complimentation groups(A-I) and "variant type" which is considered to have a defect in the post-replication repair. We have already reported that the proteinaceous factor(s) (A-factor) with molecular weights of 90 Kd and 160 Kd in HeLa cell extracts restores specifically the defect of group A cells. We have also detected the similar activities in the crude extracts prepared from various species and tissues--- human placenta,bovine thymus,bovine liver,mouse Ehrlich's ascites tumor cells,oocytes of Xenopus laevis. Thus far we have purified the A-factor from bovine liver several thousand fold. It's molecular weght is 10-30 Kd under a denaturated condition. We will discuss some other natures of the A-factor.

B 9

TRANSFECTION OF HUMAN OR MOUSE MICROCELLS CORRECTS ABNORMAL RESPONSE TO MITOMYCIN C-INDUCED CHROMOSOME ABERRATION OF FANCONI'S ANEMIA FIBROBLASTS. Michihiro C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Fanconi's anemia fibroblasts were fused with microcells obtained from human anueploid cell lines established from lung cancer and neuroblatoma. In metaphases of mirocell-FA heterokaryons examined 2 - 7 days after fusion, the high rate of mitomycin C (MMC)-induced chromosome aberrations was found to be fully corrected. A metaphase plate with a single chromosome from a microcell origin also showed a reduced rate of MMC-induced chromosome aberration. However, the correcting chromosome is rearranged and has not yet been definitively identified. Metaphases contained prematured condensed chromosomes (PCC) also corrected FA cells. These results constitute a first step toward chromosomal assignment of a gene required for the repair defect in FA cells.

B 10

ヒト子宮頸癌細胞へのヒト１１番染色体導入による造腫瘍性の抑制。児井稔・押村光雄（神奈川がんセ・研・細胞）。 SUPPRESSION OF TUMORIGENICITY BY INTRODUCTION OF HUMAN CHROMOSOME 11 IN HUMAN CERVICAL TUMOR CELL LINES. Minoru KOI and Mituo OSHIMURA (Lab.Cell Biol.,Kanagawa Cancer Res.Inst.,Yokohama,Kanagawa)

ヒト子宮頸癌由来細胞，HeLa 細胞の造腫瘍性発現は，正常ヒト細胞由来の１１番染色体の導入によって抑制されることが最近 Stanbridge らによって示された。我々は，正常ヒト１１番染色体の存在が HeLa 細胞以外のヒト子宮頸癌細胞の造腫瘍性を抑制するかを検討する目的で，pSV2-neo 遺伝子の挿入されているヒト１１番染色体を１本有するマウス A9 細胞から SIHA 及び C-4Ⅱ，へ微小核融合により１１番染色体の導入を行った。
現在までに，SIHA より２クローン，C-4Ⅱ より３クーロン得たがヌードマウスにおける造腫瘍性が抑制された。
以上のことは，これらの子宮頸癌の発生過程にはヒト１１番染色体上の正常遺伝子の消失あるいは不活化が関与していることを示唆する。

B 11

ヒト横紋筋肉腫細胞へのヒト１１番染色体導入による造腫瘍性の抑制，押村光雄・児井稔（神奈川がんセ・研・細胞）． SUPPRESSION OF TUMORIGENICITY BY INTRODUCTION OF HUMAN CHROMOSOME 11 IN HUMAN RHABDOMYOSARCOMA CELL LINES. Mituo OSHIMURA and Minoru KOI (Lab.Cell Biol.,Kanagawa Cancer Res.Inst.,Yokohama,Kanagawa)

ヒト横紋筋肉腫の発生過程には，ウイルムス腫瘍と同様に，１１番染色体短腕上の遺伝子の欠失あるいは突然変異による不活化に加え，優性正常対立遺伝子の消失が重要な役割をもつことが示唆されている。
我々は，ヒト正常培養線維芽細胞（MRC-5）に優性遺伝子マーカーである pSV2-neo 遺伝子をカルシュウム沈殿法によりトランスフェクションし，細胞融合および微小核融合を過て pSV2-neo 遺伝子が挿入しているヒト１１番染色体を１本有するマウス A9 細胞を得た。その細胞からヒト横紋筋肉腫細胞株 A204 および RD に再び微小核融合によりヒト１１番染色体導入を試みた。現在までに得られた５クローンのヌードマウスにおける造腫瘍性を検討したところ，造腫瘍性の抑制が認められた。
以上のことは，「横紋筋肉腫の発生過程には ヒト１１番染色体上の正常対立遺伝子の消失が重要である」という仮説を強く支持する。

B 12

GENETIC POLYMORPHISM OF HUMAN RIBONUCLEASE (RNase 1) Koichiro KISHI, Wataru SATO, Toshihiro YASUDA, Yasuyuki KAWAI and Keiko MIZUTA (Dept. Legal Med., Fukui Med. Sch., Fukui)

One of the human urinary ribonucleases(RNases) was isolated and purified by means of a series of column chromatographies in a homogeneous state obtained by SDS-polyacrylamide gel electrophoresis. The enzyme was designated as RNase 1, a glycoprotein with a molecular weight of around 14,000. Rabbit antibody to the purified RNase 1 reacted with human urine and sera, as well as the purified RNase 1.

The genetic polymorphism of serum RNase 1 was studied by polyacrylamide gel isoelectric focusing, followed by immunoblotting using antisera specific for RNase 1. Two common phenotypes, RNASE1 1 and RNASE1 1-2, were easily recognized using this technique. The homogeneous phenotype, RNASE1 1, was constituted by four major bands with different pI values, and the heterogeneous phenotype, RNASE1 1-2, was presumed to represent a mixture of each of the homogeneous phenotypes; however the other homogeneous phenotypes, RNASE1 2, was not detected in our samples. Family studies are in agreement with an autosomal codominant transmission of the two alleles. Population studies indicate that the frequencies of the *RNASE1*1* and *2* alleles are 0.988 and 0.012, respectively.
(Am. J. Hum. Genet. in press)

B 13

A CASE OF A WOMAN WITH MULTIPLE ABORTIONS DUE TO BLOOD GROUP M INCOMPATIBILITY WHO DELIVERED A LIVE CHILD BY PLASMAPHERESIS.

Ken FURUKAWA, Tamiko NAKAJIMA (Dept. Legal Med. Gunma Univ., Maebashi), Tadahisa KOGURE (Dept. Legal Med. St. Marianna Univ., Kawasaki), Mitsunori YOSHIDA, Takao FUKAISHI, Yoshito IBUKI and Masao IGARASHI (Dept. Gyne. Gunma Univ., Maebashi)

The case of a woman who had had three abortions caused by anti-M was reported in 22nd of this Congress. During all three previous pregnancies anti-M antibodies produced in her serum gave a titer of 1:4096 by antiglobulin technique. The incomplete antibody was increased up to 1:8192 after an artificial termination of pregnancy and 14 weeks abortion. In July 1985, the patient reported that she was 2 months pregnant. Plasmapheresis were carried out from 2 months' gestation and 2 times in every two weeks. Anti-M titer decreased 1:256 to 1:512. She delivered a female baby by induction of labor at 35 weeks. The mother was group O N CDe/CDe. The husband's cells were shown to be group O M cDE/cDE and the baby was O MN CDe/cDE. At birth, anti-M antibody titer of the mother's serum was 1:256. No other antibody against husband's cells was demonstrated. The child's cells had a strongly positive direct anti-globulin reaction and her serum contained IgG anti-M antibody. Photothreapy was carried out and the child has developed normally.

B 14

GENETIC ANALYSIS OF GRAVES' DISEASE.

Hirotoshi SHINAGAWA, Akinori KIMURA, Ryoko OHKUBO, Hirofumi NISHI, Kazunori URABE, Michio YASUNAMI, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka), Hajime TAMAI, Sunao MATSUBAYASHI (Dept. Psychosom., Kyushu Univ., Fukuoka), Kanji KUMA (Kuma Hosp., Kobe), Kohoichi SUZUKI, Hideo MATSUMOTO(Dept. Legal Med., Osaka Med. Col., Takatsuki)

Blood samples from 94 patients with Graves' disease(64 are microsomal Ab positive and 30 are negative) were studied for HLA, C4, C2, Bf, Gm and Km allotyping. The incidence of some HLA class I antigens(A2, Cw3, Bw46, Bw54) and class II antigens (DRw4.1, DQWa) were increased in patients. This suggested that two haplotypes (A2-Cx46-Bw46 and Bw54-DRw4.1-DQWa) were assocated with Graves' disease, but the incidence of the latter haplotype was increased only in patients without microsomal Ab. In C4 allotyping C4AQ0 and B2 were increased. C4B2 is in linkage disequilibrium with HLA-Bw46, but C4AQ0 is not linked to any increased HLA antigens. No association with C2, Bf or Gm allotype was observed. Km allotyping shows remarkably different distribution between Ab positive and negative groups.

Our data indicated that the disease susceptibility to Graves' disease was primarily associated with HLA class I or class III region, but a distinct clinical type of the disease (Ab negative)was also associated with a distinct type of Ig light chain and HLA class II antigens.

B 15

POLYMORPHISM OF HUMAN COMPLEMENT IN TAKAYASU DISEASE.
Fujio NUMANO (Dept. Int. Med., Tokyo Med. Dent. Univ., Tokyo), Koichi SUZUKI and Hideo MATSUMOTO (Dept. Legal Med., Osaka Med. Univ., Osaka)

Recent studies on Takayasu disease (pulseless disease) have focussed on the roles of genetic factors in its pathophysiological condition. Our family and population studies on HLA antigens revealed that a haplotype of HLA Aw24-Bw52-Dw12 has been associated with genetic factors involved in Takayasu disease. In these studies, genetic polymorphism in human complement was analysed in 79 patients of Takayasu disease. There were no statistically significant differences in frequencies of C2, C3, BF, C6 and C7 allotypes between patients with Takayasu disease and 215 people who served as their controls. However, a statistically significant high frequencies of C4A2 (χ^2: 27.2, $p<0.01$) and C4BQ0 allotype (χ^2: 8.7, $p<0.01$) were demonstrated in these patients. Furthermore, all patients carrying C4A2 were found to be associated with C4BQ0. Also patients with HLA Bw52 were strongly associated with C4A2BQ0. These data suggest some genetic factor(s) may contribute to the pathophysiological condition of Takayasu disease, which is strongly associated with complotype of HLA Bw52-C4A2BQ0.

B 16

POLYMORPHISM OF THE A SUBUNIT OF THE COAGULATION FACTOR XIII: EVIDENCE FOR THE SUBTYPES OF THE FXIIIA*1 AND FXIIIA*2 ALLELES. Koichi SUZUKI, Kiyoshi MATSUI, Shigenori ITO, Kiyoshi FUJITA, Hideo MATSUMOTO (Dept. Legal Medicine, Osaka Medical College, Osaka)

Agarose gel isoelectric focusing (pH5-6.5) revealed heterogeneity characterized by a narrow or broad type of the electrophoretic band of the A subunit of plasma coagulation factor XIII (FXIIIA). Isoelectric focusing in polyacrylamide gels supplemented with 2M urea could clearly discriminate three different patterns in each of the two homomeric dimers, FXIIIA 1 and FXIIIA 2. These patterns can be explained by the existence of two codominant subtypes in each of the two common alleles, FXIIIA*1 and FXIIIA*2. These subtypes are termed FXIIIA*1A, 1B, 2A, and 2B, respectively. In random population samples of Japanese all the possible phenotypes deduced from the four codominant alleles were observed except the FXIIIA 2A homozygote. This hypothesis is compatible with the segregation study on several family samples. The frequencies of the four alleles were calculated in 433 unrelated Japanese at 0.2748 for FXIIIA*1A, 0.6201 for FXIIIA*1B, 0.0069 for FXIIIA*2A, and 0.0982 for FXIIIA*2B. Two independent mutations in the ancestral gene and one intragenic recombination might be involeved in the genesis of the four subtypes. The data obtained in this study will contribute to disputed paternity cases and to anthropological surveys.

B 17

SPECIFIC MONOCLONAL ANTIBODY FOR TESTIS OF C57BL/6 MICE. Osamu MIKAMI, Hiromi SAKAMOTO, Yoshihiro YAMAMOTO, Tomoko HASHIMOTO, Masafumi HANDA and Jun-ichi FURUYAMA (Dept. Genet.,Hyogo Coll. Med., Nishinomiya)

Female mice of the C57BL/6 strain were immunized with a homogenate from mice testis and Freund's complete adjuvant, then spleen cells and P3U1 cells were fused in the presence of polyethylene glycol. Growing cells were screened by ELISA using the pellet from testis homogenate as antigen and the cultured media as the antibody source. After cloning, two hybridomas producing IgM, were obtained. Specificity of monoclonal antibody (MoAb) in ascitic fluid was investigated by indirect immunofluorescence, using frozen sections. MoAb 1A1 reacted specifically with the cytoplasm of spermatogonia and spermatocytes but not with spermatozoa. Testicular antigen (TA) was prepared by tissue sonication, followed by gel filtration in Sephacryl S-200 columns. TA was detected in the void volume by ELISA, then analyzed by SDS-PAGE and further investigated by Western blotting. Immuno-staining of membrane filters revealed a broad area within the 45,000 and 205,000 daltons range. TA in the void volume was treated with proteolytic enzymes to determine its nature, but no changes were observed after Western blotting. However, after keratanase treatment fading of the staining was detected. From these data, we suppose that the epitope recognized by the MoAb 1A1 is within the keratan sulfate moiety.

B 18

POPULATION GENETIC STUDIES OF NATIONAL MINORITY GROUPS IN HAINAN ISLAND, CHINA: POLYMORPHISMS OF COMPLEMENT SYSTEMS. Keiichi OMOTO, Katsushi TOKUNAGA, Zhimin ZENG (Dept. Anthropol., Univ. Tokyo, Tokyo), Ruofu DU, Hong ZHAO, Feng JIN (Inst. Genet., Acad. Sinica, Beijing), Chuanshu DU and Zhi jung LUO (Dept. Med. Genet., Sun Yat-sen Univ., Guangzhou)

Polymorphisms of complement systems were examined in the blood samples from 275 Li, 61 Miao and 120 Hui individuals of Hainan Is., China. In BF, only alleles F and S were detected. In C6, alleles A, B and B2 were found in three groups, along with M91 found only in Li at low frequency. In C7, alleles 1, 2, 3, 4 were all found, the C7*4 frequency ranging from a low of 0.002 in Li to a high of 0.095 in Miao. Further, three possibly new rare variants were found in Li, tentatively called C7 L1, L2 and L3. In C8, alleles A, B and B1 were found in all three groups, except that B1 was not found in Li. Relatively marked inter-group differences in allele frequency were noted in BF and C4 systems. Overall distribution of alleles were similar to those of Han Chinese and in Japanese, although Hui tended to diverge from Miao and Li.

B 19

CLINICOGENETIC STUDY ON APLASTIC ANEMIA. Norio FUJIKI, Yoshihiro KOHLI, Mikio HIRAYAMA, Motozumi NOMURA, Tatsuro MUTOH, Masao NAKANAGA, Yoshi-yuki OHNISHI, Fukiko SATOH, Masanori DOCHIN, Shigeyuki NAKAZAKI (Dept. Int. Med., Fukui Med. Sch., Fukui) and Kazuo MANO (Nagoya First Red Cross Hosp., Nagoya)

In our first survey on aplastic anemia, 151 random cases and 28 cases with familiar occurrence among 1594 cases collected on 1972 by the study group, were checked. Second survey was performed on 167 cases with aplastic anemia and 107 matched cases with leukemia.

The frequency of consanguinity, sex, age and birth order were followed through Koseki records, and familiar occurrence of severe anemia, malignancy and heart anomalies were checked by members of the study group. There have noticed high frequency of consanguinity, familiar occurrence of these items. The genetic susceptibility would be played an important roles participating with some autosomal recessive and polygenic components.

We applied Falconer's method to calculate the heritability of aplastic nemia. It was calculated 47 ± 9 %, instead of 37 ± 9 % for leukemia.

The polymorphic traits were surveyed on 200 blood samples, it revealed significant high Hp^1, Gpt^1 and P^D for aplastic anemia.

Due to the genetic susceptibility participating with environmental agents, such multifactorial diseases might be possibly prevented by careful avoidance of environmental exposures.

B 20

AMINO ACID SUBSTITUTIONS OF THREE HAPTOGLOBIN VARIANTS. Jun-ichi ASAKAWA and Chiyoko SATOH (Dept. Genet., RERF, Hiroshima)

A total of 23,326 children of atomic bomb survivors in Hiroshima and Nagasaki were examined for plasma haptoglobin by starch gel electrophoresis. Variants were encountered in 44 children. Haptoglobin is composed of α and β chains. O'Farrell's two-dimensional electrophoresis analysis with haptoglobins purified from the variants revealed that 20 cases of the variation occurred in β chains. These 20 variants were classified into three classes (1BHR1, 2BNG1, 2BHR1) by their mobility, and the amino acid substitutions were examined. Haptoglobin, purified from plasma (2~3 ml) by affinity chromatography and carboxymethylated, was separated into β and α chains by gel filtration on TSK gel 3000 SW in the presence of 8M Urea. Digestion of the β chain with Lysyl Endopeptidase followed by reversed-phase chromatography yielded 16 fragments. Each fraction was hydrolyzed in hydrochloric acid and the amino acid analysis was carried out using PITC method. The digest of β chain from the 2-1BHR1 variant, which is very similar to the Marburg variant, was separated into 17 fractions. An abnormality was observed in fraction 9 (β231-240). The variant peptide had the amino acid substitution Asp→His at position 236 of β chain. In the same way, we showed that 2BNG1 resulted from a Glu→Lys substitution at β159 and 2BHR1 from an Arg→Pro substitution at β125.

B 21

ABNORMAL HEMOGLOBINS IN FUKUYAMA DISTRICT: Kazuo HIDAKA[1], Iwao IUCHI[1] and Goro IWAKAWA[2] ([1]Kawasaki Med. Sch., Kurashiki,[2]Natl. Fukuyama Hosp., Fukuyama)

The survey of hemoglobinopathy in Fukuyama district has been conducted in the computer identified individuals totaling 27,165 from April, 1985 to October, 1987. Twenty-three abnormal hemoglobins(abn. Hb) were detected(20/26,464: HbA variant, 3/701: HbF variant). The incidence of abn. Hb is, therefore, 1/1323 or 0.075% for HbA variant indicating 2.5 times high frequency and 1/232 or 0.43% for HbF variant, 4 times high in Japan. Of the 23 variants, I) ten were Hb Ube-2(α68 Asn → Asp) and II) the interesting variants were Hb St. Lukes(α95 Pro → His) and a new variant of Hb Fukuyama(β77 His → Tyr). III) The other interesting one was detected electrophoretically fast moving Hb. It seemed to be β chain anomaly because of high proportion(46.3%). However, IEF of PCMB treated HbX demonstrated clearly an α chain anomaly. The structural analysis of this α^X chain is under way. IV) Three HbF variants were 1) Hb F-Koyobuki($^{A_I}\gamma_{16}$Glu → Gly, 14.6% of total HbF), 2) a new HbF variant, Hb F-Fukuyama($^{A_T}\gamma$ 43 Asp → Asn, 36%) and 3) Hb F-Forest Park($^{A_T}\gamma$ 73 Asp → Asn, 35.8%). The genotypes of them were supposed from the amount of γ chains of HPLC as 1) G. $^{X}A^{I}$/G. A^{I}, 2) G. $A^{I}/^{X}A^{T}$.— and 3) G. $A^{I}/^{X}A^{T}$.— in the aforementioned order. The analyses of all the rest of Hb variant are in progress.

B 22

THE EFFECT OF CONSANGUINEOUS MARRIAGES ON THE FREQUENCIES OF MALFORMATIONS. Yoshikazu KUROKI, Yoshitsugu SUGIO and Kiyoshi IMAIZUMI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

Kanagawa Birth Defects Monitoring Program(KAMP) was established in 1981 as the first population-based birth defects monitoring program in Japan. The program covers about 50 per cent of the total births in Kanagawa prefecture. The total number of births investigated in the pro gram during the years 1981-1986 was 246,348. Data on the effect of consanguineous marriages on the frequencies of surface congenital malformations was presented. The frequency of consanguineous marriages was 0.27 per cent. The incidence of total surface malformations was 3.33 % in consanguinity. The value was 3 times as much as that in non-consanguinity(1.07 %, $p < 0.005$). In addition, the proportion of multiple malformations was markedly increased. Significantly increased isolated malformations were cleft lip with or without cleft palate, preauricular skin tags, pigmented nevi, congenital hydrocephaly, cleft palate and polydactyly. The incidence of multiple malformations was generally increased, but no special malformation syndrome occurred significantly. The results of this study suggested: (1) participation of some recessive genes in dysmorphogenesis, (2) multiple malformations could be incuced by homozygousity in non-linked double or triple loci, (3) consanguineous marriages would increase liabilities to isolated malformations.

B 23

RECONSTRUCTION OF PHYLOGENETIC TREES FOR HUMAN POPULATIONS BY THE NEIGHBOR-JOINING METHOD

SAITOU Naruya (Department of Anthropology, Univ. Tokyo)

Recently, Saitou and Nei (1987; Mol. Biol. Evol. 4:406-425) proposed a new method of phylogenetic tree reconstruction from distance data and named it the neighbor-joining (NJ) method. This method uses the principle of minimum evolution, and does not depend on the assumption of the constant rate of evolution. It was shown by computer simulations that the NJ method is generally better than other methods in finding the correct topology. In this study, the NJ method was applied to genetic distance data of various human populations, and reconstructed trees were compared with those obtained by other methods. From Nei and Roychoudhury's (1982; Evol. Biol. 14:1-59) data for 18 populations, trees were reconstructed by UPGMA, the distance Wagner method, and the NJ method (the latter two methods produce unrooted trees). UPGMA clusters Mongoloid and Caucasoid populations, whereas Caucasoid populations are placed between Negroid and Mongolid populations when other two methods are used. Except for Caucasoid, all other four races (Amerind, Australoid, Mongoloid, and Negroid) each constitute monophyletic groups in the tree reconstructed by the NJ method. A similar grouping of populations is also observed for Bowcock et al.'s (1987; Gene Geography 1:47-64) data based on 47 RFLP markers.

B 24

STUDIES ON DOWN SYNDROME –ANALYSIS OF MATERNAL AGE IN JAPAN–.

Yurika NOSAKA, Akira TONOMURA (Dept. Cytogenet. MRI .Tokyo Med. Dent. Univ., Tokyo) and Takehiko KURITA (Konodai Hosp., Natl. Center Neurol. Psychiat., Chiba)

The present study was undertaken to examine whether the pattern of the maternal age distributions of Down syndrome with standard trisomy 21 has changed with time or not. The data on maternal ages were obtained from 1,474 patients born in 1955~1979 who had been outpatients of the Pediatric Clinic of the Konodai Hospital. For analyses of the data on maternal age, annual vital statistics for all Japan were used as controls. The mean maternal ages for each of the patient's groups, classified by five-year's intervals, were consistently higher than those for each of the control groups. However, the disparity in age between the two distributions has been decreasing from 4.1 years in the year 1955~59 to 2.2 years in the years 1975~79. The relative incidence of Down syndrome increased almost exponentially with advancing maternal age. The rising patterns of the relative incidences for old mothers (above 40 years) and young ones (under 24 years) were particularly remarkable with time. These patterns may attribute to the recent increase of the prevention of threatened abortion, particularly for the first pregnancy in old mothers and decrease of spontaneous abortion in young ones.

B 25

GENETIC EPIDEMIOLOGY AND PREVENTION OF DUCHENNE MUSCULAR DYSTROPHY

Masao KANAMORI(Dept. Health Statistics, The Institute of Public Health, Tokyo), Newton E. Morton(Dept. Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York), Keiko Fujiki(Dept. Ophthalmology Juntendo Univ., Tokyo), and Kiyotaro KONDO(Dept. Public Health, Hokkaido Univ., Sapporo)

Duchenne muscular dystrophy(DMD) is caused by a sex-linked gene with a high mutation rate, located in Xp21. Classical segregation analysis was performed on 651 male probands in 597 families with DMD collected from 20 of 25 National Institutions for Muscular Diseases during 1979 - 1982. The expected proportion of sporadic cases is 1/3. An equal mutation rate in egg(9.2×10^{-5}/gamete/generation) and sperm (10.9×10^{-5}) is estimated. The incidence and prevalence among males were estimated to be 29.2×10^{-5} and 6.7×10^{-5}, respectively. These results indicated no difference from the patterns of DMD in western countries. About its preventive system, it is necessary to build up the medical registry for DMD pedigree, and to detect the patient by CPK laboratory test as early as we can.

B 26

MORTALITY RATE OF AMYLOIDOSIS IN JAPAN: SECULAR TRENDS, EFFECT OF MARITAL STATUS, AND SEASONAL AND GEOGRAPHICAL VARIATIONS.

Yoko IMAIZUMI (Inst. of Population Problems, Ministry of Health and Welfare, Tokyo)

The amyloidosis death rate in Japan was analyzed using Japanese vital statistics for 1969-1985. The amyloidosis death rate increased gradually year by year for both sexes. The changing patterns in mortality might be explicable by a constantly improving ascertainment of amyloidosis. The amyloidosis death rates for each sex among the four marital categories indicated similar values except the widow group for males and the single group for females. The geographical variations in the amyloidosis death rate were also observed with the highest death rate in Kumamoto and Nagano prefectures for both sexes. The difference in the amyloidosis death rate was not significant among the six occupational categories of the head of the household. The mean age at death from amyloidosis gradually increased year by year for both sexes, though the age was 11-23 years shorter for males and 20-25 years shorter for females than that of the general population.

B 27

RELATIONSHIP BETWEEN BLOOD URIC ACID LEVEL AND PERSONALITY TRAITS.

Shuichi OOKI(1), Akio ASAKA(1), Kazuaki YAMADA(2) and Takamasa SAITO(1).
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2) Dept.Pub.Hlth., Sch.Med., Showa Univ.,Tokyo

The present study deals with the relationship between blood uric acid level(BUAL) and human behavior. Subjects were 37 monozygotic twins(MZ) and 7 dizygotic twins(DZ), aged from 18 to 45. BUAL was standardized using regression lines separately for males and females. The distribution of standardized BUAL corresponded well with the theoretical curve of normal distribution. The intraclass correlation coefficient for BUAL was 0.370($p<0.05$) for the 37 MZ twins, but the correlation was not significant for the 7 DZ twins. These findings suggest that BUAL is genetically controlled. In the evaluation of the correlation between standardized BUAL and 12 subscales of YG-test, significant correlation was observed with respect to "lack of agreeableness" and "rhathymia". Since these two personality traits include "activity", our findings are consistent with the generally accepted results that persons with high BUAL are more active and energetic than those with low level.

B 28

HISTORY OF THE ADULT DISEASE IN TWINS. Akio ASAKA, Kazuaki YAMADA, Takamasa SAITO and Shuichi OKI (Sch. Health Sciences, Univ. Tokyo Fac. Med., Tokyo)

Subjects were 104 monozygotic twins (MZ) and 15 dizygotic twins (DZ), aged from 18 to 50. Mailed questionnaire was used to get informations about the history of the adult disease, including diabetes mellitus, hypercholesterolemia, hypertension, peptic-duodenal ulcer, hepatic disease, cerebrovascular disease, coronary heart disease, kidney disease, respiratory organ disease, hematic disease and malignant neoplasia. Among 104 MZ, 7 pairs showed complete discordance for the history of kidney disease and other 7 pairs for the history of respiratory organ disease. It was conspicuously observed that there were 13 pairs with complete discordance for the history of peptic-duodenal ulcer. Among these 13 pairs, for example, a clearly different life history was seen in one 45-year-old female twins. The twin who had been suffered from the ulcer had experienced more stressful life events, if compared to the co-twin without the history of the ulcer. YG test indicated that the twin had less active, more labile and more nervous character than the co-twin.

B 29

CONGENITAL ANOMALIES OBSERVED IN AUTOPSY CASES OF HUMAN FETAL AND NEONATAL TWINS. Yukio SATOW, Hiroshi HORI (Dept. Genet. Res. Inst. Nucl. Med. Biol. Hiroshima Univ. Hiroshima), Takayosi IKEDA (Dept. Pathol. Nagasaki Univ.) and Naomasa OKAMOTO (Miyazaki Med. Coll. Miyazaki)

To observe how heredity and environmental factors affect the occurrence of anomalies in human beings, congenital abnormalities were studied in four of the 14 cases of conjoined twins (thoraco-abdominal-pagus etc., monozygotic) and 30 of the 251 cases of disjoined twins, selected from among some 10,000 human fetal and neonatal autopsy cases. Hypoplasia of toes, double ureter, accessory spleen, excessive lobulation of lung, and left diaphrogmatic hernia were found in both of the disjoined twins, and anencephaly, tetralogy of Fallot, coarctation of the aorta, persistent left superior vena cava, accessory spleen, incomplete lobulation of lung, abnormal bifurcation of the great artery, accessory adrenals, and cleft lip, in either of the twins. Although it cannot be said that the hereditary background is stronger in the abnormalities developing in both of the twins or that environmental factors had a greater effect on the abnormalities observed in either of the twins, it is remarkable that anencephaly and some cardiac abnormalities are observed only in either of the twins. We wish to offer the above results as a reference for use in the analysis of the factors involved in congenital abnormalities.

B 30

SIMILARITY DIAGNOSIS OF ORO-FACIAL REGION ON MONOZYGOTIC TWINS —— STATISTICAL EXAMINATION OF THE CONSISTENCY OF MEASURED VALUES. Shiroshi KIKUCHI (Dept. Oral Surg., Fac. Med., Teikyo Univ., Tokyo)

Monozygotic twins are considered to have same quality for their genetic characteristics. However, the consistency of indexes of oro-facial region has not examined closely. The author tried to examined the consistency of dental indexes (four measured values of dental arches) of twins using "errors-in-variables" linear regression model (Tango,1987). Four indexes are transversal and sugittal sizes of the maxilla (A,B) and mandible (C,D). Results of analysis were as follows: transversal sizes indicated that the sloop was 1.116(A) or 1.185(C) and the intercept was -6.778(A) or -10.339(C); sugittal sizes indicated that the sloop was 0.795(B) or 0.876(D) and the intercept was 8.269(B) or 4.453 (D). And these rates were completely included in 95% confidence interval (BOOTSTRAP). These results revealed that the data of each twin were consistent at any variable and that the data did not shifted systematically. Thus the consistency of these indexes of oro-facial region was statistically proved.

B 31

BIOLOGICAL AND PSYCHOSOCIAL FACTORS RELATED TO THE FORMULATION OF PROBLEM DRINKING BEHAVIOR

Kazuaki YAMADA (Dept.Pub.Hlth., Showa Univ., Tokyo) and Akio ASAKA (Dept.Mental Hlth., Sch.Med., Univ.Tokyo, Tokyo)

Twin study was carried out to investigate the correalation of biological and psychosocial factors related to the formulation of drinking behavior. Subjects, composed of 37 monozygotic twins(MZ) and 9 dizygotic twins(DZ), were graduates of the high school affiliated to Tokyo university. First, factor scores such as 'sensitivity to aldehyde', 'sensitivity to ethanol', 'motivation of drinking', 'some problem drinking behavior', were prepared as the evaluating scales according to principal component analysis of general population's data(N=1829). Intraclass correlation coefficients were calculated. Some of the highest values in MZ were of 'sensitivity to aldehyde'(0.731), 'social problem'(0.775). Next, percent deviations of those scores were calculated. By multiple regression analysis, 'the difference of motivation' was selected as most effective factor to explain the difference of drinking behavior. This result suggests the relative importance of psychosocial formulating factors of drinking behavior compared with bio-genetic ones.

B 32

ANALYSIS OF 10 YEARS EXPERIENCES IN THE GENETIC COUNSELING CENTER.

Koji OHKURA (Dept. Human Genet., Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo)

The Genetic Counseling Center of the Japan Family Planning Association, Inc. were established under the Family Planning Special Counseling Project of the Ministry of Health and Welfare in 1977.

In the past 10 years, the center received 10,723 contacts and nearly 2500 counseling has been done. The clients spread in allover Japan but predominantly Tokyo area. Female clients were double of male in number, and there 2 peaks (21-35 and over 51 years) in age distribution were observed.

As the sourse of information hospitals and health centers (38%) and printed informations (34%) were very important. Majority of clients recived counseling before marriage or pregnancy (90%). Counseling session were done within one hour (60%) and 95% of them finished the counseling with one session. Most of consultand (73%) was the child of clients. Some 388 cases were regard to general problems on consunguinity without particular disorders. Kinds of abnormalities or diseares were counted morethan 300 while subtypes were grouped.

Careful analyses of there data will be very important bases of future practices of genetic counseling in many respects in Japan.

B 33

PRESENT AND PROBLEMS OF GENETIC COUNSELLING AT TORANOMON HOSPITAL.
Kodo SATO, Masako HORIGUCHI, Muneaki KIMURA, Eiki SO, Takahisa KOJIMA, Manabu TAMURA, Kaoru UNO, Ikuko YOKO-O (Dept. Obstet. Gynecol. Toranomon Hospital, Tokyo)

Recent 500 cases of genetic counselling carried out between 1986-1987 at Toranomon Hospital were analysed and discussed, especially comparing with the 599 cases at Tokyo University Hospital between 1976-1981. At the both institutes, the chief counsellor was among the authors. Total number of visiting clients increased 4 times, mainly due to the increase of clients related to advanced maternal age and those with previous child of minor chromosomal abnormalities. Out of the 500 cases, 266 cases had antenatal diagnosis by chorionic villus sampling(6), amniocentesis(235), fetal blood sampling(2) and ultrasonography(9). Antenatal diagnosis becomes a more important tool for genetic counselling and network system is suggested to be required for efficient utilization of highly specialized diagnostic methods for genetic counselling in Japan.

B 34

FAMILY HISTORY STUDY OF AFFECTIVE DISORDER.
Hiroshi YONEDA, Tohru ISHIDA and Toshiaki SAKAI (Department of Neuropsychiatry, Osaka Medical College, Osaka)

The present investigation concerns a family history study in 82 consecutive patients with predominantly affective symptoms. The object of this study is to confirm that this group of patients are genetically heterogeneous.

The clinical material consisted of 82 patients who had been admitted to the Department of Neuropsychiatry, Osaka Medical College during the period from 1965 to 1974. We divided all the cases into two groups on the basis of symptomatology i.e. the typical and the atypical type with slight clouding of consciousness and/or schizophrenic symptoms, and compared the intrafamilial variation of pathological traits. The cases of the atypical type showed a significantly higher incidence of schizophrenic traits (44.4%) than that of the typical type (3.1%). Next we divided the clinical material into two groups, one with mood incongruent pathological experiences and the other without these symptoms. The group with mood incongruent pathological experiences showed a higher incidence of intrafamilial schizophrenic traits. These results indicate that affective disorders are genetically heterogeneous.

B 35

SURVAY OF GENERAL KNOWLEGE ABOUT HEREDITY. Mitsushiro KIDA (Dept. Pediat., Teikyo Univ., Tokyo)

The survay was conducted by questionnare method. A total of 714 people were polled, including 27 doctors, 175 medical students, 39 ordinary citizens, 419 woman junior college students, and 54 nursery school teachers. There were 158 males and 552 femals. The guestionnair consisted of five questions. Of the 458 general citizens and junior college students surveyed, those not having any specialized medical knowlege, 121, or 26.4%, responded that they thought they had bad genes. By contrast, only 17(6.6%) of the 256 medical school students, nursery school teachers and doctors, gave the same answer. Knowlege about mutation was widespread, as an average of 93% in each group. Many respondents, 44% of the total, indicated that they did not know the meaning of evalution. Approximately 20% in every group had no knowlege of meiotic division and non-disjunction, the caus of Down's syndrome. A correct understanding of dominant and recessive inheritance was shown by 40% overall (28~44%). An inability to distinguish between heredity and hereditaly disease was seen in 40%(48 of 120) of the older respondents.However,only 21.1%(143 of 594) of the younger genereation students were unable to make a distinction. What was suprising was that 23% of the medical school students, could not distinguish between chromosomal anomaly and genetic disease.

Japan has already entered the era of biotechnology at this time. It is essential for the future survival of the human race that everyone have a correct understanding of human genetics and congenital anomalies.

B 36

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS SPECIFIC TO JAPANESE AND/OR MONGOLIANS. Akihiro SUZUKI, Yasunari SEKIGUCHI, Yoshito ASHIMURA, Hidemitsu KUROSAWA, Yuuichi NAKAGAWA, Lois MULLIGAN, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (National Children's Medical Research Center)

Restriction fragment length polymorphisms (RFLPs) are a powerful form of genetic marker. They have been proved to be quite useful in family and population studies for genetic diseases as well as for cancer research. Almost all the RFLP probes that are so far available, were established after screening DNA samples isolated from Caucasians. Therefore, they do not necessarily reveal polymorphisms in different ethnic groups. We have analyzed 60 DNA samples isolated from unrelated Japanese individuals using such RFLP probes. Among 40 loci we analyzed, 5 did not show any polymorphisms in the Japanese population. An RFLP probe for 19p13-cen revealed a pattern distinctive from that in Caucasians.

B 37

DNA POLYMORPHISMS IN THE β-GLOBIN GENE CLUSTER IN KOREANS.
Koji SHIMIZU (Inst. Develop. Res., Aichi Pref. Colony, Kasugai), Kyung Sook PARK (Dept. Biol., Sung-Shin Women's Univ., Seoul), Keiichi OMOTO (Dept. Anthrop., Univ. Tokyo, Tokyo).

DNA polymorphisms in the β-globin gene cluster were examined in healthy Koreans at seven restriction sites (Hinc II 5' to ε; Hind III in Gγ; Hind III in Aγ; Hinc II in ψβ1; Hinc II 3' to ψβ1; Ava II in β; Bam HI 3' to β). Major haplotypes were [+-----+], [+----+-], and [+----++]. Seven were homozygous for [+-----+] (f=0.326), and four, for [+----+-] (f=0.246), among 66 individuals. As to subhaplotype 5' to the δ-globin gene, 25 homozygotes for [+----] (f=0.615), and 3, for [-+-++] (f=0.213), were identified. Only one chromosome with [-++-+], which should carry the AγT-globin gene, was observed among 60 chromosomes examined. It is suspected that the frequency of the AγT-globin gene in Koreans must be low as in the case of Chinese. On the other hand, it is known that its frequency in Japanese is rather high. The distribution of the β-globin gene frameworks in Koreans tells us that the genetic relationship between Koreans and Chinese may be closer than that between Koreans and Japanese.

B 38

DNA POLYMORPHISMS IN THREE JAPANESE POPULATIONS
Satoshi HORAI, Kenji HAYASAKA and Ei MATSUNAGA (Lab. Human Genet., Nat. Inst. Genet., Mishima)

Nuclear DNA was isolated from 180 Japanese, 60 each from Aomori, Shizuoka, and Okinawa populations, and 30 foreigners of four races, Whites, African Blacks, Asians, and a Papuan. RFLP of the DNA was analyzed by southern hybridization using probes derived from different chromosomes. Among the probes used, pHs-49 (HRAS-1) and pAW-101 (D14S1) detected the restriction patterns controlled by alleles which were specific to each race. Using these two probes, we analyzed RFLP among 180 Japanese derived from the three populations. Among the two probes, pHs-49 showed highly polymorphic restriction patterns. In particular, in the digestions by Taq I, 5, 7 and 8 restriction patterns were observed in Aomori, Shizuoka, and Okinawa populations, respecitvely. These patterns were assumed to be controlled by 5 alleles, each with the fragment sizes of 3.85, 3.7, 2.6, 2.5 and 2.3 kb. The allelic frequencies in the three populations were similar to each other. This observation is in contrast to the results obtained from the analysis of mitochondrial DNA polymorphism which exhibited local differences.

B 39

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs) IN A JAPANESE POPULATION (1). Norio TAKAHASHI, Mieko KODAIRA, Junko KANEKO, Noboru KOSAKA and Chiyoko SATOH (Dept. Genet., RERF, Hiroshima)

Our laboratory is currently exploring the feasibility of studying mutation rates at the DNA level. An important prerequisite to developing an efficient approach is to define the type and frequency of natural variation in the probes that might be used. Although there are many reports of RFLPs in other populations, there are not so many reports concerning RFLPs in Japanese populations. We report here results of a study on RFLPs in a Japanese population from Hiroshima using Southern blotting with probes which can examine 6 gene regions (PGK1, HP, HPR, CALC1, CA2, COL1A2) and 4 arbitrary DNA regions (D3S2, D7S8, D9S1, D17S1). For these regions, the presence of RFLPs have been reported in other populations. DNA samples were obtained using proteinase K treatment from cell lines established from B-lymphocytes from 49 unrelated Hiroshima individuals by Epstein-Barr virus transformation. Restriction enzymes were those used to detect RFLPs in other populations. RFLPs were detected in the Japanese population in all of the ten DNA regions. Significant differences were observed in RFLP frequencies in the COL1A2, HP (EcoRI site polymorphism), HPR, PGK1, D3S2, D7S8 (TaqI site polymorphism), and D17S1 genes between Japanese and other populations.

B 40

CARRIER DETECTION OF HEMOPHILIA A BY DNA ANALYSES. Nobuhiro SUZUKI, Takeshi NAGAO, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (Kanagawa Children's Medical Center and National Children's Medical Research Center)

Hemophilia A is a genetic disease which is caused by a defect in the factor VIII gene located at Xq28. DNA analyses will be useful to detect carriers of hemophilia A in affected families and in prenatal diagnoses. To measure polymorphic information contents in the Japanese population, we first analyzed 60 DNA samples isolated from unrelated Japanese individuals using two intragenic probes (BclI & BglI) as well as two extragenic probes (TaqI/St14 & BglII/DX13). The St14 probe revealed a different polymorphism in Japanese from those in Caucasians. We succeeded in carrier detection in 9 out of 12 families at risk for hemophilia A.

B 41

RFLPs OF T CELL ANTIGEN RECEPTOR GENES IN SLE AND NORMAL CONTROL.
Megumi HIIDA, Hidetoshi KANEOKA, Fumitaka MORITO, Akihide OHTA and Masaya YAMAGUCHI (Dept. Int. Med., Saga Med. Sch., Saga).

We analyzed the restriction fragment length polymorphisms (RFLPs) in Systemic Lupus Erythematosus (SLE) using cDNA probes for T cell antigen receptor α , β and γ chain genes in order to elucidate the genetic factor(s) contributing to the development of immunological abnormality in SLE. We studied 25 normal individuals and 35 SLE patients. DNAs were digested with 6 different restriction enzymes (EcoR I, Hind III, BamH I, Pst I, Pvu II and Bgl II) and no RFLPs were seen when hybridized with C_α and C_γ probes. But when using C_{β_2} probe, Hind III and Bgl II-digests showed RFLPs. In two of them, only Hind III-digest showed significant difference between patients and controls. Thus, 13kbp band, frequently seen in normal population, was less seen in SLE patients ($p < 0.01$). The relative risk of suffering SLE for a person without 13kbp band is approximately 5. This difference might account for one of the genetic factors concerning the development of immunological disorders in SLE.

B 42

APPLICATION OF APOLIPOPROTEIN AI-CIII GENE POLYMORPHISM TO A PATIENT WITH APOLIPOPROTEIN AI DEFICIENCY.
Naoko HATTORI, Yosikazu HIASA*, Hisako YANAGI, Juichi SATOH, Kimiko YAMAKAWA, Takaaki OKAFUJI, Yasuko YAMANOUCHI, Kenji YUZAWA, and Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Tsukuba; *Dept. Cardiol., Komatsushima Red Cross Hosp., Komatsushima)

Apolipoprotein AI (apo AI) is the major protein constituent of HDL. In order to determine the allelic frequency of RFLPs in the apo AI-CIII gene region, 43 unrelated healthy Japanese were analyzed by Southern blot hybridization using apo AI cDNA which was kindly gifted from Dr. Humphries. Seven common haplotypes could be identified by five RFLPs which were detected with Msp I, Sac I, Pst I, Xmn I, and Taq I. Using these five RFLPs and their haplotypes, we analyzed the apo AI gene in a female patient with apo AI deficiency associated with a marked reduction of HDL and coronary heart disease. No gross alteration of apo AI-CIII genes were detected. However, she was homozygous for all five RFLPs with the haplotype 11112. Her parents were first cousins. The probability homozygous for the haplotype 11112 was estimated to be 0.03 in the child whose parents are first cousins. In addition, low HDL cholesterol levels were observed in her two sisters as well as in all of her three children. The data suggest that the apo AI deficiency is autosomal recessive and is caused by a mutant apo AI gene.

B 43

ALLELE FREQUENCY OF c-Ha-RAS-1 IN THE JAPANESE POPULATION AND APPLICATION TO THE PATERNITY TESTING. Terue HARUMOTO[1], Kiyoshi MATSUI[1], Shigenori ITO[1], Koichi SUZUKI[1], Yuuji MATSUO[2], Tokiko MIYAZAKI[1] and Hideo MATSUMOTO[1] ([1]Dept. Legal Med.; [2]Dept. Transfu., Osaka Med. Col., Takatsuki)

The allele frequency distribution of polymorphic region of c-Ha-ras-1 was analyzed in the Japanese population. DNA samples from 320 unrelated individuals were digested with HinfI and hybridized with the probe of flanking region of c-Ha-ras-1 gene. The common alleles previously reported as a1(2.6kb), a2(3.2kb), a3(4.0kb) and a4(4.3kb) were observed, but the frequency of a2 allele was significantly low in the Japanese population. However, the frequency of distribution was in a good accordance with those expected from the Hardy-Weinberg equilibrium. The frequencies of a1, a2, a3 and a4 alleles were 0.8469, 0.0016, 0.0969 and 0.0547, respectively. Pedigree analysis revealed that the four alleles were codominant and inherited in the Mendelian fashion. The results were applied to the paternity testing. The probability of paternity exclusion was calculated as 12.7 %.

B 44

DNA POLYMORPHISM OF THE LDL RECEPTOR GENE IN JAPANESE: II. PIC AND LINKAGE DISEQUILIBRIUM IN 7 RFLPs. Takaaki OKAFUJI, Kimiko YAMAKAWA, Naoko HATTORI, Yukio IWAMURA*, and Hideo HAMAGUCHI (Dept. Hum. Genet., *Dept. Microb., Univ. Tsukuba, Tsukuba)

Mutation in the gene for the LDL receptor give rise to familial hypercholesterolemia(FH). RFLPs of the LDL receptor gene are useful markers for the genetic analysis of FH. In order to study RFLPs of the LDL receptor gene in Japanese,we digested DNA samples from 56 unrelated indivisuals with restriction enzymes, and analyzed by the Southern blot hybridization using the LDL receptor cDNA probe pLDLR-3 gifted kindly from Dr. D.W. Russell.

Six previously reported RFLPs (detected by AvaII,ApaLI,PvuII,NcoI,and PstI), and new RFLPs (detected by TaqI) were obserbed in a Japanese population. The gene frequency and PIC were TaqI(p=0.69, PIC=0.33) AvaII(0.79, 0.28), ApaLI(5')(0.66, 0.35), PvuII(0.88, 0.19), NcoI(0.55, 0.37), PstI(0.83, 0.24), and ApaLI(3')(0.52, 0.37). Among 21 two-RFLPs-site combinations, 13 showed linkage disequilibrium ($p<0.01$ or $p<0.05$). Extremely high frequency of recombination was observed between the two Alu repetitive sequences in the intron 15. The PIC estimated based on the haplotypes frequency was 0.83. The haplotypes identified by 7 RFLPs are useful genetic markers for FH in Japanese.

B 45

RFLP ANALYSIS IN THE FAMILIES WITH Duchenne muscular dystrophy

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It has been shown that restriction fragment length polymorphism (RFLP) analysis is a powerful method for identifying the chromo some locations of the defective genes in genetic disorders such Duchenne muscular dystrophy(DMD). Subcloned DNA segments,pERT 87 -],8,15 were donated by L.M.Kunkel and these 3 probes were used for RFLP analysis with Southern blotting in 26 DMD families. The frequencies of common alleles that Kunkel reported were dif--ferent a little. That is to say,the reversed patterns were obta--ined in the case of the conbination of these probes and enzymes ,pERT87-1/BstN1,pERT87-8/Taq1, pERT87-15/Xmn1, and the frequency of rare allele by using pERT87-15/Taq1 was much lower than that of Kunkels report. The frequency of heterozygosity of these probes except pERT87-15/Taq1 were all 37 to 49%. This showed that these 3 probes were very useful for RFLP analysis in DMD families. The frequency of heterozygosity with the two Xmn1 sites detected by pERT87-1 and pERT87-15 was 75% (26 of 32 tested females) This suggested that the conbination of Xmn1 RFLP detected by both pERT87-1 and pERT87-15 was the most informative and useful for RFLP analysis in Japanese DMD families.

B 46

CA19-9 IN SERUM AND SALIVA SAMPLES FROM CANCER PATIENTS AND THEIR LEWIS BLOOD-GROUP PHENOTYPES. Shin YAZAWA, Ken FURUKAWA (Dept. Legal Med., Gunma Univ., Gunma), Hideaki IZAWA and Takayuki ASAO (Gunma Cancer Center, Gunma)

The frequencies of Le(a-b-), Le(a+b-) and Le(a-b+) blood-group of erythrocytes in 360 patients suffering from various cancers were 97(26.3%), 74(20.6%) and 189(52.5%), respectively. Eighteen patients showed high levels of CA19-9 in sera, even though the blood-group phenotypes of their red blood cells were Le(a-b-). Most of the patients were in the late stage of diseases. Seven of these patients (group I) were determined as Le(a-b-) from both red blood cells and saliva consistently, whereas eleven other patients (group II) secreted either Le^a or Le^b antigen in saliva and showed the expression of incompatible Lewis blood-group antigens. GDP-fucose:N-acetylglucosaminide α-(1→4)-L-fucosyltransferase was demonstrated to be present in salivas from both group I and II. These results suggest that a cancer-associated alteration of Lewis blood-group antigen expression occur in cancer patients.

B 47

EXPRESSION OF LEWIS-RELATED ANTIGENS IN HUMAN COLORECTAL TISSUES FROM PATIENTS WITH FAMILIAL POLYPOSIS COLI AND FROM NON-HEREDITARY SPORADIC CASES. Jun-ichi SOEJIMA, Masayuki SASAKI, Kenji SUGIO, Takehiko SASAZUKI (Dept. Genet., Kyusyu Univ., Fukuoka)

Expression of 7 Lewis-related antigens (Le^a, Le^b, Le^x, Le^y, sialylated Le^x, sialylated Le^x-i, and polyfucosylated Le^x) in normal mucosa, polyps and cancers from 57 patients with FPC and 42 non-hereditary sporadic cases were examined using monoclonal antibodies in immunohistochemistry. Both in FPC and non-hereditary sporadic cases, expression of Le^b, Le^y, sialylated Le^x and sialylated Le^x -i was increased paralleled with tumor progression. In normal mucosa (both proximal and distal colon), sialylated Le^x and sialylated Le^x-i were more expressed in the tissues from patients with FPC than those from non-hereditary sporadic cases. Similar high expression of Le^b and Le^y was observed in normal distal mucosa but not in proximal mucosa. In polyps, sialylated Le^x expression was correlated with the premalignant parameters of larger polyp size and severer dysplasia and sialylated Le^x-i expression was correlated with severer dysplasia both in FPC and non-hereditary cases.In conclusion, Le^b, Le^y, sialylated Le^x and sialylated Le^x -i antigens are concidered as onco-developmental antigens. Normal mucosa of FPC patients seems to get one step further to tumorigenesis than that of non-hereditary sporadic cases.

B 48

GENETIC ANALYSIS OF FAMILIAL POLYPOSIS COLI(IV): DIFFERENTIAL EXPRESSION OF c-MYC AND c-FOS GENES IN COLON TUMORS IN FPC. Kenji SUGIO, Masayuki SASAKI, Jun-ichi SOEJIMA, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka)

The expression of 8 oncogenes(H-ras, K-ras, c-myc, N-myc, L-myc, c-fos, c-fms, c-sis) and MHC class I, II genes, and the structure of 19 oncogenes were analyzed in 15 adenocarcinomas, 18 adenomas, and 18 normal colonic mucosa from 19 patients with familial polyposis coli. 1) The expression of c-myc gene was most elevated in carcinoma and moderately elevated in adenoma compared with corresponding normal colonic mucosa. In contrast, the expression of c-fos gene was markedly decreased in both adenoma and carcinoma. These characteristic expression of the genes were also demonstrated in non-hereditary colon carcinoma. 2) The expression of MHC genes showed no correlation with the stage of tumorgenesis or with the expression of myc gene family. 3) Amplification of c-myc gene was detected in one carcinoma and Southern blot analysis suggested that c-myc gene was duplicated first and the duplicated genes were amplified as a set.

These data suggested that c-myc and c-fos genes might be involved in cellular proliferation and differentiation in colonic epithelial cells.

B 49

GENETIC ANALYSIS OF FAMILIAL POLYPOSIS COLI (V) : LOSS OF CONSTITUTIONAL HETEROZYGOSITY OF COLONIC TUMORS. Masayuki SASAKI[1], Kenji SUGIO[1], Jun-ichi SOEJIMA[1], Mieko OKAMOTO[2], Michiko MIYAKI[2], Takehiko SASAZUKI[1] ([1]Dept. Genet., Kyushu Univ., Fukuoka; [2]Dept. Biochem., Tokyo Med. Science, Tokyo)

FPC(familial polyposis coli) is a genetic disorder, transmitted as an autosomal dominant trait, and is characterized by numerous colorectal adenomas and by high incidence of malignancy. The major gene for FPC was mapped to 5q and the loss of heterozygosity was observed on chromosome 5 by Bodmer et al. In this study, we searched for somatic loss of heterozygosity in colon tumors derived from patients of FPC and non-polyposis colon cancer (NPCC), by using Southern blot analysis with several DNA markers. In colon cancers from FPC, somatic loss of heterozygosity was observed on chromosome 5, 6, 12, 15 and 22 (22.2-50%). Loss on chromosome 5, 6, 12 and 22 was also observed in colon cancers from NPCC (11.1-37.5%). In adenomas from FPC, on chromosome 6 and 12. Our data revealed that the loss of constitutional heterozygosity is not the specific event on chromosome 5 and suggested two possibilities : (1) tumor suppressor gene may be located on one of the chromosomes where loss of heterozygosity was detected, (2) loss of constitutional heterozygosity may be the result of abnormal mitotic events during tumorigenesis and may not have any relation to the etiology of cancer.

B 50

Primary rearrangement of 9q34 and 22q11 in the genesis of variant Philadelphia (Ph[1]) chromosome translocations in chronic myelogenous leukemia (CML). Syuiti ABE[1], Masako MINAMIHISAMATSU[2], Takaaki ISHIHARA[2], and Motomichi SASAKI[1] ([1]Chromosome Res. Unit, Fac. Sci., Hokkaido Univ. and [2]Div. Radiat. Hazard, Natl. Inst. Radiol. Sci.)

Ph[1] chromosome is associated with about 90% of CML patients. The majority of Ph[1]-positive cases have the standard Ph[1] translocation involving chromosomes No.9 and No.22, t(9;22)(q34;q11), while the minority of cases have a variant type of Ph[1] translocations involving these two and other chromosomes (complex) or those involving No.22 and chromosomes other than No.9 (simple). We examined the localization of c-abl and c-sis oncogenes and bcr gene in nine variant Ph[1] translocations of CML by using in situ chromosome hybridization, to elucidate the formation of these unusual translocations. The in situ hybridization showed that c-abl (9q34) was translocated on Ph[1] chromosome and c-sis (22q12-q13) on one of involved chromosomes other than No.9 in all variant translocations examined. In addition, bcr (22q11) was translocated on chromosome No.9 in most of the translocations. The present findings suggest: 1) chromosome No.9 is always involved in variant Ph[1] translocations; 2) rearrangement of 9q34 and 22q11 is the primary event in the formation of variant translocations; 3) simple variant translocations are hidden complex variant translocations involving 9q34.

B 51

21 LARGE SATELLITE IN A PATIENT WITH CMMoL AND IN HIS RELATIVES.
Kunihiko HISATAKE, Toshinao YAMANO, Kouichiro IYOTA, Yukio YOSHIMOTO, Hiroshi OKAMURA, Kenzo YOSHIDA, Yukiko NUMATA, Fumitoshi OHNO (The 2nd Dept.of Intern.Med.,Kochi Med.Sch.,Kochi) and Yukimasa SHIRAISHI (The 1st Dept.of Anat.,Kochi Med.Sch.,Kochi)

A 37-year-old male was diagnosed as chronic myelomonocytic leukemia(CMMoL,MDS typeIV)with the rare symptom of systemic lymph node swelling. On admission,his peripheral blood monocyte count was 1400/μl. A few myeloid cells in bone marrow were positive for double staining by α-naphthyl butyrate and naphthol ASD chloroacetate esterase. Biopsied specimens of the cervical lymph node showed infiltration of monocytoid cells,which were positive for lysozyme staining,in the interfollicular tissue. Anti-HTLV-I andIIIantibodies were negative. Furthermore,a chromosome variation, 46,XY,21S+,was observed in all the dividing cells in his bone marrow and blood. The 21 large satellite had the same fluorescence intensity as Y chromosome stained with quinacrine mustard. It was also stained by AG's method. The same chromosome variation was found in three relatives(mother,son and daughter)who seem healthy. The chromosome variation accords with transmission as an autosomal dominant trait. In this patient,the chromosome variation and CMMoL were coincidentally observed. His healthy mother has giant platelets in peripheral blood and a high level of serum lysozyme which are characteristic manifestations related to CMMoL. The results suggest that CMMoL may be caused by this chromosome variation.

B 52

IMMUNOGENETICS OF ADULT T-CELL LEUKAEMIA III. ROLE OF CLASS-I MHC ANTIGENS IN THE NK SENSITIVITY OF LEUKAEMIC T-CELLS.
Hisamitsu UNO, Hitoshi MATSUOKA, Kiyohide KAWANO, Shuichi TOGAMI, Kazunori TSUDA (2nd Dept. Med., Miyazaki Med. Coll., Miyazaki)

The altered expression of HLA class I antigens on the peripheral leukaemic T-cells of patients with ATL was demonstrated by microcytotoxicity assay, flowcytometry, and by staining individual leukaemic T-cells with anti-HLA class I monoclonal antibody by the method of immunochemistry. We examined the biological significance of altered expression of class I antigens on leukaemic cells in patients with ATL, as concerned NK sensitivity of the leukaemic cells. Peripheral lymphocytes from patients with ATL or healthy control did not show NK activity against HTLV-I bearing T-cell lines wtih increased expression of class I antigens. We selectively decreased the class I antigens of cell lines by treating them with pH3 buffer. NK sensitivity of the cell lines revealed an inverse correlation with the expression of class I antigens on their surface. This inverse correlation was also observed by treating target cell lines with anti-HLA class I antibody.
The effector cells involved in the NK cytolysis were demonstrated to positive for CD16, HNK-1 or CD3, indicating that they were NK cells.
The observation suggested an inverse correlation between class I antigen expression on HTLV-1 bearing cells and their NK sensitivity.

B 53

REGIONAL ASSIGNMENT OF RETINOBLASTOMA (RB1) AND ESTERASE D (ESD) TO 13q14.13. Kouji NARAHARA, Kiyoshi KIKKAWA, Yoshiharu WAKITA, Hiroshi NANBA, Hiroshi KIMOTO (Dept. Pediatr., Okayama Univ., Okayama) and Ryozou KASAI (Asahigawa Jidoin, Hosp. Handicapped Child., Okayama)

In Human Gene Mapping 8, RB1 and ESD have been assigned to 13q14.1. The recently isolated cDNA for ESD, however, was found to hybridize to 13q14.2 or 13q14.3 instead of 13q14.1. To solve the discrepancy between the cytogenetic and molecular studies, we examined chromosomes and gene dosage effects for ESD in a case with 13q-, where an unilocal retinoblastoma of well-differentiated type was recognized in the right eye. High resolution banding analysis showed breakpoints on 13q14.13 and 13q21.32. ESD phenotype in the patient, father and mother was 1, 7-1 and 2-1, respectively. ESD activities of red blood cells were all within the normal range in the three individuals. Tumor cells whose chromosome constitution was identical to that in the somatic cells, on the other hand, was heterozygous for the loci: on isoelectric focusing another main band was seen more anodal than ESD 1 band. These results showed that ESD lies proximal to RB1 in the junctional region of bands 13q14.13 and 13q14.2. The ESD changes in the retinoblastoma cells may suggest that either submicroscopic deletion of the normal chromosome 13 or perturbed transcription of RB1 and ESD to mRNA is involved in the tumorigenesis.

B 54

c-MYB ONCOGENE IN A NEWLY ESTABLISHED HUMAN T CELL LINE WITH del(6q). Michiko OKADA, Masako SAITOH, Kura KUBOTA, Yoshiko NOMURA (Chromosome Lab., Shiseikai Dai-Ni-Hosp., Tokyo), Naotoshi KANDA[1], Naohiko MASUDA[2], Hideaki MIZOGUCHI[2] ([1]Dept. Anat. and [2]Div. Hematol., Tokyo Women's Med. Coll., Tokyo), Fuyuki ISHIKAWA (Dept. Intern. Med., Tokyo Univ., Tokyo)

We established a T cell line, KT, with del(6)(q21q25) from a patient with lymphoblastic non-Hodgkin's lymphoma. From the comparison of the surface phenotype and the karyotype of fresh and cultured cells, KT cell was proved to derive from the lymphoma cell. As the deleted #6 segment corresponds to the reported band to which c-myb oncogene was mapped, we studied whether or not rearrangement of this oncogene occurred, using Southern hybridization technique. DNAs from KT cell and from a placenta(control)were digested with the single or combined use of EcoRI, BglII, SacI, HindIII and BamHI, and hybridized to the 2.0-kb(CO044) and 2.6-kb(CO045) EcoRI digested c-myb probes which were provided by Japanese Cancer Research Resources Bank. No change was observed in KT cell DNA. And Northern hybridization analysis showed the expression of this oncogene, in accord with the lineage characteristic.

B 55

STRUCTURAL ABNORMALITIES IN THE HUMAN N-myc ONCOGENE. Masao YAMADA, Yuuichi NAKAGAWA, Hidemitsu KUROSAWA, Yasuhide HAYASHI, Keiko YAMAMOTO and Yasuo NAKAGOME (National Children's Medical Research Center and Saitama Children's Medical Center)

N-myc is a member of myc oncogene family. N-myc gene amplification was described in several childhood cancers, but other abnormalities are not known. We found several cases in which the structure of the human N-myc gene was changed. A rhabdomyosarcoma obtained by a surgical operation from an untreated patient, contained 5-6 fold amplified gene as well as a single copy of rearranged N-myc gene. The promoter region was replaced with an exogenous DNA fragment of unknown origin. Metastasizied tumors of the same patient contained the amplified N-myc in the same extent but did not contain the rearranged structure. A DNA sample isolated from the placenta of normal delivery showed additional DNA bands in Southern hybridization when probed with N-myc. It was accounted for by amplification of a new member of the myc related genes. Several neuroblastoma cell lines which had extensively amplified N-myc also contained rearranged structure.

B 56

RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF THE HUMAN N-myc GENE. Yuuichi NAKAGAWA, Hidemitsu KUROSAWA, Lois MULLIGAN, Yutaka NAKAHORI, Masao YAMADA and Yasuo NAKAGOME (National Children's Medical Research Center)

Using Southern blot hybridization with N-myc probes, we observed two restriction fragment length polymorphisms in the Japanese population. Gene frequencies were analyzed in the normal population and neuroblastoma patients. We also demonstrated that RFLP was useful to analyzed amplified allele. Most of these results are in press in the ONCOGENE.

B 57

NONRANDOM KARYOTYPIC CHANGES IN IMMORTAL AND TUMORIGENIC SYRIAN HAMSTER CELLS INDUCED BY DIETHYLSTILBESTROL

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Kiyo SASAMOTO Yuko SHINTAKU Akira YAJIMA (OBGYN, Tohoku Univ.)
Mitsuo OSHIMURA (Kanagawa Cancer Center Res. Institute)
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Treatment of Syrian hamster embryo cells with diethylstilbestrol(DES) resulted in the induction of immortal cell lines which progressed and formed tumors in nude mice. Four independently treated cell lines were analyzed cytogenetically at several passages during neoplastic progression. The immortal cell lines at early passages had no structural abnormalities but did have numerical changes. For example, gain of chromosome 11 was found in all immortal cell lines, and gain of chromosome 19 was found in two of four cell lines. Tumorigenic cells showed not only a variety of numerical abnormalities but also structural abnormalities. Loss of a sex chromosome and gain of chromosome 19 were found in six of seven tumors. Gain of chromosome 11, which was found in all immortal cell lines, disappeared in five of seven tumors. Structural abnormalities involving chromosomes 2 and 3 were found in three of seven tumors. Many marker chromosomes were also found in the tumors.

B 58

GENE CONVERSION IN STEROID 21-HYDROXYLASE GENES. Kazunori URABE, Akinori KIMURA, Tomohisa IWANAGA, Michio YASUNAMI and Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka)

Congenital adrenal hyperplasia due to 21-hydroxylase (21-OHase) deficiency is an autosomal recessive defect in adrenal steroidogenesis. There are two 21-OHase genes, A and B, in the HLA class III region in man. The 21-OHase B gene is active, while the 21-OHase A gene is a highly homologous psudogene. To understand the molecular basis of the disease, we cloned and sequenced a 21-OHase B gene from a patient with 21-OHase deficiency. The nucleotide sequencing revealed that there was a nucleotide change (C →T) which brought a premature termination codon in the 8th exon and the sequences around this change were identical to that of 21-OHase gene. These data suggest that a gene conversion of an active 21-OHase B gene to the pseudogene (21-OHase A gene) causes 21-OHase deficiency in this patient. Besides, the gene organization of HLA class III region in normal individuals was studied by Southern blot analysis. The HLA-B7-DR1 haplotype was shown to have triplicated C4 and 21-OHase genes that may be generated by an unequal crossover and a part of 21-OHase A gene was converted to 21-OHase B gene in two HLA haplotypes, HLA-B44-DRw13 and HLA-Bw46-DRw8.

B 59

DNA ANALYSES OF PATIENTS WITH PRADER-WILLI SYNDROME (PWS). Tsutomu KAMEI,*Tatsuro KONDOH, Hidefumi TONOKI, Junichi HAMABE, Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet.,*Dept. Pediat., Nagasaki Univ., Nagasaki), Tomoko HASEGAWA (Div. Med.Genet., Shizuoka Child. Hosp., Shizuoka)

PWS is associated with 4 different karyotypes: del(15);t(15;?);inv dup(15); normal. We studied gene dose effects in 7 typical PWS cases with del(15), 2 with t(15q;15q) where deletion exists, 2 cases of atypical PWS with or without deletion and in their parents, using cloned DNAs, p3-21 and #34 which were derived from inv dup(15), as hybridization probes. Densitometric analysis revealed that, in all the typical or atypical cases with del(15), HindIII-digests gave 1 copy of both p3-21 and #34 fragments, while the parents, both cases of t(15;15) and 1 atypical case all had two copies of the fragments. Thus, the patient with cytogenetically visible deletion did not always show deletion in the DNA sequences used in this study, indicating that the probes may not be derived from but be linked to the PWS gene. Parental origins of del(15) in 2 patients were traced using the AluI/p3-21 RFLP as a marker. In both cases, deleted chromosomes originated in the paternal meiotic error, being consistent with the result of our previous study.

B 60

VARIETY OF GENE MUTATIONS IN 21-HYDROXYLASE DEFICIENCY (21-OHD). Tatsuro KONDOH,*Tadashi MATSUMOTO,*Norio NIIKAWA, Masaaki YOSHIMOTO, Yoshiro TSUJI (Dept. Pediat.,*Dept. Hum. Genet., Nagasaki Univ., Nagasaki), Kaoru SUZUMORI (Dept. Gyn. Obst., Nagoya City Univ., Nagoya), Noritaka IWATANI (Dept. Pediat., Kumamoto Univ., Kumamoto), Masahiro TETSUOH (Dept. Gyn. Obst., Yame City Hosp., Yame)

DNA analyses of 10 patients with 21-OHD in 8 unrelated families were performed using 21-OH gene cDNA (pC21/3c) and C4 gene cDNA (pAT-A) as hybridization probes. In one patient, TaqI digests hybridizing to pC21/3c lacked both 3.7kb and 2.3kb fragments which correspond to the 21-OH B and the 21-OH A genes, respectively. With KpnI and pAT-A, she also lacked a 3.6kb fragment. These findings indicate a deletion from 3' side of the A gene to 5' side of the B gene. Though another 2 patients showed similar deletion patterns with TaqI-pC21/3C hybridization, when the digests of other enzymes were hybridized to each probe, one of the 2 patients had all the fragments consistent with those in normal controls, indicating a gene conversion mechanism as a cause of the disease of this patient. The mutation in the other patient was either deletion or gene conversion. Since no deletion patterns were detected in the remaining 7 patients, their mutations may be a base substitution or by other mechanisms.

B 61

COMPLEXED GLYCEROL KINASE DEFICIENCY: DELETION ANALYSES AND CLINICAL STUDIES OF 5 PATIENTS. Tadashi MATSUMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki), Masaaki YOSHIMOTO (Dept. Pediat., Nagasaki Univ.), Koichi YANO (Dept. Pediat., Asahikawa Med. Coll., Asahikawa), Kenji FUJIEDA (Dept. Pediat., Hokkaido Univ., Sapporo), Masato TSUKAHARA (Dept. Pediat., Yamaguchi Univ., Ube), Shigeki TOYOTA (Dept. Pediat., Kagawa Med. Sch., Kagawa)

Complexed glycerol kinase deficiency (CGKD) is a rare X-linked contiguous disease. Five patients with CGKD were studied clinically, and cyto- and molecular-genetically. All patients had DMD-like muscular dystrophy and adrenal hypoplasia (AH) in addition to GKD. Four of the 5 patients were mentally retarded and had growth deficiency. High-resolution GTG-banding showed a micro-deletion in Xp21 region in all patients and mothers. Southern hybridization with various cloned DNAs located around Xp21 also revealed a DNA deletion in the genome of all patients. Though deletion ranges differ among patients, a segment commonly involved in deletion was confined between L1-4 and J66-HI sequences. A comparison study of manifestations of the patients with their DNA deletions suggested that that there exists a certain gene as a mental and/or growth determinant (M/GD) in between ERT87-8 and J66-HI sequences, and that the genes around Xp21 are arranged in order of cen-OTC-DMD-M/GD-GK-AH-pter.

B 62

ORIGIN OF THE DISEASE CAUSING GENES FOR JAPANESE TYPE APRT DEFICIENCY Naoyuki Kamatani, Shoko Kuroshima, Chihiro Terai, Kazuo Kawai and Kusuki Nishioka (Inst. Rheum., Tokyo Women's Med. Col., Tokyo)

More than 2/3 families with DHA lithiasis reported in the world have been Japanese. Approximately 80% of all the Japanese families with this disease synthesize a mutant adenine phosphoribosyltransferase (APRT) having a reduced affinity to a substrate, 5-phosphoribosyl-1-pyrophosphate and increased heat-stability. We have presented a model of the genotypes for various APRT deficiencies in which the Japanese type homozygotes have a genotype of APRT*J/APRT*J. We have provided evidence that APRT*J is a disease causing allele deriving from a common ancestorand widely distributed among Japanese. We analyzed Taq I RFLP of human APRT gene on 9 families with the Japanese type APRT deficiency as well as control subjects. Two types of alleles (each producing 2.7 kb and 2.1 kb fragments respectively) were detected as reported among Caucacians (Stambrook et al. Somat. Cell Mol. Genet. 10,359). Frequencies of the two types of alleles among control subjects were 50% and 50%. All of the 9 patients from 9 separate families with the genotype of APRT*J/APRT*J were homozygous having only 2.7 kb-associated alleles. These data indicate that APRT*J originated from a mutation in a 2.7 kb-associated allele in an ancestor of Japanese probably after the separation of Japanese and Caucacian ancestors.

B 63

THE ANALYSIS OF CHROMOSOMAL PROTEINS BY USING MONOCLONAL ANTIRODIES.
YASUFUMI KANEDA, MARI WATAYA-KANEDA, KEIKO KATO, TSUYOSHI UCHIDA
(Institute for Molecular and Cellular Biology, Osaka Univ., Osaka)

We isolated more than 20 monoclonal antibodies against nucleus to analyze the structure and function of nucleus and chromosomes. Here we report one of the antibodies, M108 (IgM) whose antigen reveals in nuclear envelope, perichromosomal region and cytoplasmic vesicles. Immunofluorescence and cell cycle studies showed that M108 recognized nuclear envelope in G1, whole nucleus in S and perichromosomal region in G2 and M phase. Moreover in G2, cytoplasmic vesicles were detected by this antibody. Such vesicles were rich in the cytoplasm of micro-cells. Protein blotting analysis showed that M108 reacted dominantly with a 40kD protein in the cytoplasmic fraction and chromosomes. The antigen in perichromosomal region disappeared after the treatment of DNase I (40 μg/ml). Our results suggest that about 40kD protein in perichromosomal region and cytoplasmic vesicles has important rolls in the formation and breakdown of nuclear envelope.

C 1

A DISPERMIC CHIMERA WITH DYSGERMINOMA.
Hisao TAKIZAWA[1], Isao NAKAMURA[1], Yutaka HIRASAWA[1], Takashi FUJIKURA[1], Takeshi MATSUDA[2], Michiko HIROSE[3], and Zenichi OGITA[3] (Dept. Legal Med.[1] and Anat.[2], Inst. Wakan-yaku[3], Toyama Med. & Pharm. Univ., Toyama), Kazumasa IKAWA, Hiroshi ISHIKAWA, Katsumi FUJITA and Keiji YAMAMOTO (Takaoka Municipal Hospital, Toyama)

A 12 years old female patient suffering from abdominal tumor was proved to be a dispermic chimera. 92% of her red cells were group A_1, PGM_1 1A, PGD AC and 8% of them were group B, PGM_1 1A-2A, PGD A. 90 cells out of 110 cultured lymphocytes were normal 46 XY and 20 cells were normal 46 XX. She secreted B and H substances at normal secretor range and small amount of A substance in her saliva. The *A*-transferase level found in her serum was about 40% of those of group A_1 individuals and in her urine was 6%. The both of *B*-transferase levels in her serum and urine were in normal range. Her hairs had B antigen and PGM_1 phenotype of their roots was 1A-2A. The cells of hair root were X chromatin-positive and Y body negative. Her nails had lesser A antigen and potent B antigen. Major epithelial cells of her buccal mucosa expressed B antigen and minor cells expressed A antigen. The appearances of X chromatin and Y body in the buccal cells were rather similar to those in females. The surgically resected abdominal tumor was her right uterine adnexa with malignant transformation, whose histological diagnosis was dysgerminoma. The tumor cell nest and epithelial cells of ductal structure expressed A antigen, whereas the endothelial cells of blood vessels did B antigen.

C 2

日本人のGc型の変異型．大上　治・中村茂基・山下ケサ子・越智順子・小原義宏
阿部和枝（東女医大、法医）．THE DISTRIBUTION OF THE GC VARIANTS IN JAPANESE.
Osamu OHUE, Shigeki NAKAMURA, Kesako YAMASHITA, Junko OCHI, Yoshihiro OBARA and Kazue ABE (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Immobiline等電点電気泳動によりGc変異型の検討を行った。検出方法はpH 4.75~5.30のImmobilineゲルを用い、電気泳動は電極をゲル上に直接置き、3500V、unlimited mA、10W、4℃で一晩行った。泳動後、蛋白固定し、Coomassie Brilliant Blue R-250で染色を行った。東京在住健康人1166名について検討したところ従来より報告されている3種のcommon allele(Gc*1S、Gc*1F、Gc*2)と、8種のvariant allele(陰極からGc*1C4、Gc*1C2、Gc*2A4、Gc*1C35、Gc*1A2、Gc*1A3、Gc*1A8、Gc*1A9)に加えて新たにGc*1A30、Gc*1A1、Gc*1C9が観察された。また、Gc*1C35は湯浅らの報告している頻度より高い値で認められた。Gc*1C35はその後 Kambohらにより、Australiaの原住民の集団から遺伝的多型性があることが報告されている。従来のIEFでの確認の結果、Gc*1C9の検出は比較的容易であるが、Gc*1C2、Gc*2A4、Gc*1C35、Gc*1S、Gc*1A1、Gc*1Fに関しては各PIが近接しているため、その検出は困難であると思われ、見落とされている可能性もあると考えられる。特に Gc*1C35については今後、地理的勾配の解明のために、さらに日本人および近隣諸集団についての調査が望まれる。

C 3

GENETIC POLYMORPHISM OF HUMAN C1R IN THE JAPANESE POPULATION.
Shigeki NAKAMURA, Osamu OHUE, Katsunori AKIYAMA and Kazue ABE (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Genetic polymorphism of the C1R subcomponent of human complement component C1 was investigated in neuraminidase treated EDTA plasma samples from 440 healthy Japanese individuals living in Tokyo by means of thin layer polyacrylamide gel isoelectric focusing(PAGIEF) in the presence of 8.0M urea followed by electroblotting with enzyme immunoassay. PAGIEF band patterns of C1R in 440 Japanese subjects were classified into six common and five rare allotypes, and these were considered to be controlled by three common and three rare alleles. In these alleles two common alleles were identical to C1R*1 and C1R*2 reported by Kamboh and Ferrell(1986). Other new alleles were tentatively designated C1R*3 C1R*4, C1R*5 and C1R*6, respectively. The results of the family studies suggested that the genetic model for C1R polymorphism assumed autosomal codominant Mendelian inheritance. The allele frequencies were estimated as C1R*1=0.4216, C1R*2=0.3602, C1R*3=0.2068, C1R*4=0.0091 and C1R*R(C1R*5, C1R*6)=0.0023, respectively. The distribution of allotypes fitted the Hardy-Weinberg equilibrium. It is interesting that the Japanese population has a large genetic variation in C1R compared with U.S. white and U.S. black.

C 4

C7 POLYMORPHISM IN JAPANESE: NEW ALLELE C7*8. Nori KOMATSU, Akira KIDO and Masakazu OYA (Dept. Legal Med., Yamanashi Med. Univ., Yamanashi-ken)

The polymorphism of C7 was investigated in neuraminidase-treated sera from 513 unrelated Japanese individuals using isoelectric focusing followed by an electroimmunoblotting technique. Eight common phenotypes C7 1, 2-1, 2, 3-1, 3-2, 4-1, 4-2, 4-3 and five rare variants were identified, one of which was determined as the type 6-1. Three variants exhibited the same pattern and looked like the type 1, but the anodal double and middle bands of this type stained more intence than the corresponding bands of the type 1. The pedigree analysis of one of the probandi confirmed the genetic transmission of a new allele C7*8, and the above variant type was expressed as C7 8-1. The remaining variant was thus phenotyped as C7 8-3. The allele frequencies were: C7*1 = 0.8314, C7*2 = 0.0926, C7*3 = 0.0380, C7*4 = 0 0331, C7*6 = 0.0010 and C7*8 = 0.0039. It is of anthropological significance that in Japanese and Chinese C7*2, C7*3 and C7*4 occur with polymorphic frequencies while in Europeans the incidence of variants seems extremely low.

C 5

IMMUNOGENETICAL STUDY FOR MITE RELATED BRONCHIAL ASTHMA.
Hitoshi MATSUOKA, Hisamitsu UNO, Kiyohide KAWANO, Syuuichi TOGAMI, Kazunori TSUDA (Dept. 2nd. Med., Miyazaki Med. Col., Miyazaiki)

The allergen Dp2 was separated from Dermatophagoides pteronyssinus which was one of major allergens for atopic bronchial asthma, and its molecular weight was approximately 15,000. There were no association between any HLA class II antigens and Dp-related asthmatics with high Dp2 specific IgE titer and/or high lymphocyte proliferative response to Dp2. Dp2 induced lymphocyte proliferation was observed only in patients. We examined this response under recombination of T cells and macrophages derived from high and/or low responders to Dp2. This response was macrophage dependent, and not HLA class I antigens but HLA-DRantigens on macrophages were important for inducing this response. When macrophages were treated with anti HLA-DR antibody, this response was inhibited. But when it was treated with the same antibody before incubation with T cells, this inhibition was not observed. So HLA-DR antigens, which appeared after incubation with T cells, might be important for inducing this response. And macrophage derived from low responder to Dp2 could induce this proliferation, when each HLA-DR antigens were identical, but macrophage from high responder failed to induced this response to T cells derived from low responder. It might be suggested that T cells restricted this Dp2 induced response.

C 6

Phylogenetic analysis of human mitochondrial DNA types.
Shinji HARAHARA (Dept. Legal Med., Univ. Tsukuba, TsuKuba),
Naruya SAITOU, Momoki HIRAI (Dept. Anthropol., Univ. Tokkyo, Tokyo),
Shogo MISAWA (Dept. Legal Med., Univ. Tsukuba, Tsukuba) and
Keiichi OMOTO (Dept. Anthrop., Univ. Tokyo, Tokyo)

Phylogenetic relationship of human mitochondrial DNA (mtDNA) types were analyzed among a total of 885 individuals in 15 populations. The data analyzed in the present study were from Johnson et al. (1983), Wallace et al. (1985), Bonné-Tamir et al. (1986), Brega et al. (1986a), Brega et al. (1986b) and Harihara et al. (1987). In these studies, four enzymes, AvaII, BamHI, HpaI and MspI, were commonly used to detect RFLP and mtDNAs of all the individuals were classified into 57 types by combining the enzymes' digestion patterns. In an unrooted tree drawn by the maximum parsimony method to connect these types, two groups, African and non-African, were depicted. In the non-African group, there were several clusters derived from the most frequent type (type 1) and some of them were composed of types found in particular populations. Phylogeny of populations was also analyzed by calculating genetic distances. In the UPGMA tree based on them, two African populations were located quite far from others.

C 7

RFLPs IN HUMAN EGF RECEPTOR GENE.
Shinobu GAMOU and Nobuyoshi SHIMIZU (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo)

Recently, the human epidermal growth factor receptor (EGFR) gene was found to be homologous to the v-erbB oncogene of avian erythroblastosis virus. The involvement of abnormal EGFR gene expression in tumor development and/or progression has been suggested. To further determine the structure and organization of the EGFR gene, we analyzed restriction fragments of DNAs from normal lymphocytes and several tumor cells. We found four common restriction fragment length polymorphisms (RFLPs). One was detected as 6-7 kb segments in the EcoRI digests, 9-10 kb segments in the HindIII digests, and as 9-10 kb segments in the PstI digests. These RFLPs were detected with the BamHI fragment (1.0 kb, probe 1) of the EGF receptor cDNA pE7 (2.4 kb) and showed linkage disequilibrium. Southern blot analysis and genomic EGFR gene analysis allowed us to estimate that EcoRI and HindIII RFLP sites are within a 10 kb region. The fourth type of RFLPs did not show linkage disequilibrium to the other three RFLPs and was detected as 4-5 kb segments in the HindIII digests with the 3' fragment (0.5 kb) of probe 1. The polymorphic nature of the EGFR gene will be extremely useful, not only for gene linkage analysis and mapping of chromosome 7, but the analysis of abnormal EGFR gene expression in tumor cells.

C 8

REEXAMINATION OF RFLP PROBES FOR DXS87 AND DXS88. Yasunari SEKIGUCHI, Akihiro SUZUKI, Naoaki JINNO, Masao YAMADA and Yasuo NAKAGOME (National children's Medical Research Center)

D. Shaw isolated two RFLP clones (pA13.RI & G3-1) which revealed polymorphisms for DXS87 (Xq21-q24) and DXS88 (Xq11-q21), respectively (HGM 8). GENE BANK in Japanese Cancer Research Resource Bank obtained the two clones directly from Dr. D. Shaw. At that time, he already suggested some problems with the G3-1 clone. We analyzed plasmid DNA and constructed restriction maps. Polymorphisms in BglII digests were also analyzed in the Japanese population using the clones. Our final conclusion is that the G3-1 clone in GENE BANK is no longer the original clone described by D. Shaw, but a clone related to pA13.RI. D. Shaw agreed with our conclusion. Since he could not find a correct clone of G3-1, DXS88 is no more useful.

C 9

BF SUBTYPES IN JAPANESE PATIENTS WITH IgA NEPHROPATHY AND WITH IDIOPATHIC MEMBRANOUS NEPHROPATHY. Hiroaki NISHIMUKAI (Dept. Legal Med., Ehime Univ., Ehime), Isao NAKANISHI (Kidney Disease Cent., Osaka Pref. Hosp., Osaka), Hajime KITAMURA (Dept. Immunol., Cent. Adult Diseases, Osaka) and Yoshihiro TAMAKI (Dept. Forensic Med., Med. Coll. Oita, Oita)

The subtyping of the properdin factor B (BF) was carried out by the method of polyacrylamide gel isoelectric focusing (PAGIEF; pH 3-10) and immunoblotting. Serum or EDTA-plasma samples were treated with neuraminidase prior to PAGIEF. BF F phenotype revealed by high voltage agarose gel electrophoresis (Alper et al.,1972) was separated into two subtypes named BF FA (anodal) and BF FB (cathodal). The allele frequencies for BF*FA, BF*FB, BF*S, and BF*F075, in normal controls, were 0.1408, 0.0164, 0.8404, and 0.0024, respectively. The BF subtypes in patients with IgA nephropathy (IgA-N) or idiopathic membranous nephropathy (IMN) were examined. The allele frequencies in IgA-N were BF*FA=0.2014, BF*FB=0.0208, BF*S=0.7708, and BF*F075=0.0070; and in IMN were BF*FA=0.2353, BF*FB=0.0882, and BF*S=0.6765. Significant differencies in the allele frequencies between IMN and the controls, and the significant association of IMN with BF FA subtype ($p<0.05$) and that with BF FB ($p<0.001$) were found. BF*FB may be a susceptibility allele to IMN rather than BF*FA.

C 10

AN EXPECTED DECREASE IN INCIDENCE OF AUTOSOMAL RECESSIVE DISEASE DUE TO A DECREASE IN CONSANGUINITY. Tomohiro SAITO (Dept. of Human Ecology, National Children's Medical Research Center, Tokyo)

A rapid decrease in consanguinity over the past four decades in Japan must have resulted in a decrease in the incidence of autosomal recessive disease. However, the magnitude of this theoretically known decrease has not been assessed before. We made theoretical estimates of the chronological decrease in the incidence with the modified Dahlberg's formula applying appropriate consanguinity rates, taken from published data, during the period from 1945 to 1983. A chronological decrease in the proportion of patients from first-cousin marriages among all the patients was also estimated.

The estimated decrease both in the incidence and in the proportion was substantial, particularly in disorders of low gene frequencies. Incidence of major autosomal recessive disorders was estimated to have decreased by 40 to 80% and the proportion of patients from first-cousin marriages dropped from around 40-70% to 5-15% during the period. The estimates of the magnitude of the decrease in the incidence and in the proportion seems to agree fairly well with an observed incidence of phenylketonuria obtained from the results of mass screening of the newborn and with an observed chronological decrease in the proportion in Wilson's disease in a study.

C 11

A FEMAL CASE OF DISTAL MONOSOMY 1q ASSOCIATED WITH PARTIAL ALBINISM (PEIBALDISM). Atsushi Ieshima, Takashi Mito (Div. Child Neurology, Tottori Univ. Sch. Med., Yonago) and Masashi Ando, Shigeto Kasagi and Madoka Shioda (Dept. Pediatr. Matsue National Hospital, Matsue)

A 11-year-old girl was the second product of unrelated healthy parents after 39 weeks of gestation. Birth weight was 1748 g. Multiple dysmorphisms, leukoderma and muscle hypotonia were noted at birth. Main clinical features at the age of 11 years were short stature (-4.5 SD), microcephaly (-5.9 SD), spastic quadriplegia, severe mental retardation (2-3 months level), intractable convulsions and multiple dysmorphic features including sparse hair, hypertelorism, epicanthus, ptosis, short nose, high-arched palate, short neck, sacral dimple and implantation of bilateral 3rd toes. Dermatoglyphics showed low TRC(17), simiancrease and arch tibiale. The R-and G-banded karyotype was 46,XX,del(1)(q42). Clinical manifestations were typical as a distal 9q monosomy syndrome. White forelock and leukoderma was observed on midline frontal area and ventral truncus. Piebaldism was diagnosed because of clinical course, distribution and shape of depigmented area, and pathology. The gene of Piebaldism is considered to be located on 4q12, but those may be related to distal 1q monosomy.

C 12

TORISOMY 3p25-pter IN A GIRL WITH LISSENCEPHALY AND DANDY-WALKER SYNDROME. Ikuko KONDO(Dept. of Human Genet., Univ. of Tsukuba, Ibaraki) Takao ENOMOTO, Tadao NOSE and Yutaka MAKI(Dept. of Nerusurg., Univ. of Tsukuba, Ibaraki)

We report a six-year-old female wirh trisomy 3p(3p25-pter) and monosomy 1q(1q44-qter) resulting from a maternal balanced translocation, 46, XX, t(1;3)(q44;p25). The facial appearance of the patient was strikingly similar to reported phenotypes of trisomy 3p syndrome, generally included a square-shaped face with frontal bossing, birateral temporal indentation, hytertelorism, prominent cheeks, long and prominent philtrum, protruding middle portion of the upper lip, and retrognathia. The constant facial characteristics in the partial 3p trisomy syndrome might be due essentially to a trisomy of the band 3p25 and 3p26 on the basis of previous reports. In addition to the typical clinical symptomes of trisomy 3p, our patient had lissencephaly and Dandy-Walker syndrome. These brain anomalies have not described previously in the patients with trisomy 3p, though hydrocephaly, holoprosencephaly and encepharocele have been complicated in seven patients with this syndrome. Thus, detailed examinations of brain anomalies may be helpful for study on neuropathogenesis of mental retardation in this syndrome, because all patients were severely mentally retarded.

C 13

A CASE OF PARTIAL TRISOMY 6q.
Kazumi IKAWA,Emiko NAKAYAMA,Misako WATANABE(Ishikawa Health Seavice.,Ishikawa) Shigeru MARUYAMA(Kanazawa Holy Spirit Hosp.,Ishikawa),and Tadao NOMURA(Ishikawa Childrens'Orthopedic Center.,Ishikawa)

Two-year-old male had partial trisomy 6q.

The clinical findings include short stature,mental retaldation(mild),plagiocephaly,epicanthus,exophthalmos,deformed auricle,limited movement of bilateral thumb,CHD(PS+total anomalous pulmonary venous return) and etc.

GTG and RBG banding revealed 46,XX,ins(12;6)(p11;q21q25) and 46,XX,dup(6)(q21→q25),ins(12;6)(p11;q21q25) in mother and patient respectively.

Although 9 cases of 6q trisomy have been reported,most of there include the 6q25→q27 or qter,and to our kowledge,our case is the first case of trisomy 6q21→q25.

C 14

12番環状染色体の一症例．後藤俊博，藤沢美朗，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），下辻常介，石井経康（箕面市立病院 小児科）．A Case of Ring12. Toshihiro GOTO, Yoshio FUJISAWA, Masatosi MATSUDA and Saburo OKUBO (Clin. Lab., Shionogi Co., Ltd.), Tsunesuke SHIMOTSUJI and Tsuneyasu ISHII (Dept. Pediatr., Mino City Hosp., Mino)

症例は小頭症および体重増加不良を主訴とした男児．家族歴に異常なく健常な両親（父27才，母22才）の第1子として出生した．在胎39週，正常分娩で生下時体重は2,080 gであった．生下時より哺乳力弱く発育不良で11ケ月現在，体重5,920 g（－4SD），身長67 cm（－3SD），頭囲40 cm（－4.1SD），他には両眼隔離，やや特異な顔貌が認められた．末梢血培養法による通常の染色体検査の結果，87％の細胞にring(12)が認められ，13％の細胞の細胞ではNo. 12モノソミーであった．尚，両親の核型は正常であった．さらに患児の染色体を臭化エチジウム法による高精度G－分染法とQ－分染法により分析した結果，核型は　45, XY, −12/46, XY, r(12)(p13.3 q24.33）と同定した．従ってr(12)の欠失部位は〔p13.3→p ter〕および〔q24.33→q ter〕となり 非常に微細な部分のみの欠失であると考えられた．また患児の細胞は本質的に全てr(12)を有していたと考えられ12番の欠失した細胞は分裂過程でのアーティファクトによるものと推測された．r(12)は外国で数例報告されているが本邦では報告例がない．本症例においては，r(12)の欠失部位が微細のためか臨床面の異常は比較的軽微（今後の経過観察は必要）であったものの，小頭症が特徴的であった．従って他に顕著な異常を認めない場合の小頭症は染色体検査の適応になると考えられた．

C 15

PARENTAL ORIGIN OF de novo CONSTITUTIONALLY ABNORMAL CHROMOSOMES
THE SECOND REPORT

Satoshi ISHIKIRIYAMA (Dept. Pediatrics, Hokkaido Univ. School of Medicine, Sapporo),
Tutomu KAMEI, Norio NIIKAWA (Dept. Human Genetics, Nagasaki Univ. School of Medicine, Nagasaki)

The majority of extra chromosomes in 13 trisomy and 21 trisomy orignate in nondisjunction during materal meiosis I. Many constitutionally abnormal chromosomes with aneuploidy are said to originate during materal meiosis, though many constitutionally abnormal ones without aneuploidy during pateral meiosis.

We analyzed the origin of constitutionally abnormal chromosomes in four cases with utilizing heteromorphism in quinacrine banding(QFQ). The abnormal chromosomes originated during paternal meiosis in three cases i.e., +t(13q;13q), +t(21q;21q), and t(4q;21q), though during maternal meiosis in 13q-. Adding these with four cases previously we reported and 41 cases in literature, in 24 ones with aneuploidy nine are paternal origin, and 15 ones maternal, on the other hand in 25 ones without aneuploidy, 19 paternal origin, and six maternal origin. Though there are rather small number of cases, the hypothesis may be right.

C 16

ANOTHER CASE WITH A NEWLY PROPOSED VARIANT OF CHROMOSOME 16 WITH AN EXTRA C-NEGATIVE, G-DARK SEGMENT IN THE SHORT ARM. Tadao ARINAMI (Ibaraki Prefectural Colony Hosp., Ibaraki), Takeki HIRANO (Ibaraki Children's Hosp., Ibaraki), and Ikuko KONDO (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

A chromosome 16 with an additional C-band negative, G-band dark segment in the proximal region of the short arm was found in a girl who was referred for mild growth and developmental retardation and some craniofacial minor anomalies including frontal bossing, flat occiput, depressed nasal bridge, micrognathia. No remarkable family history was noted, except for one spontaneous abortion in the reproductive history of the maternal grandmother. This chromosome was initially suspected of being pathologically significant. But family studies, in which the same chromosome was found in her phenotypically normal mother and maternal grandfather, suggested that it might be a harmless variant, as Thompson PW et al.(1987) reported. This might be one of examples that extra G-dark material of being apparently euchromatin does not contain active genes.

C 17

Familial chromosome aberration (18q+) with few clinical findings. Shizuhiro NIIHIRA, Hiroko FUJITA (Dept. of Child Health, Osaka City Univ., Osaka), Hajime OZAKI, Hitoshi FUNAMOTO, Kazuko SHINO (Dept. of Pediatrics, Momoyama citizens' hospital, Osaka)

We report five patients with three additional bands attached to the long arms of the 18th chromosome in one family all with normal mentality. The proband is an 8-month-old boy with micrognathia, low-set ears, and dextrocardia. He was found to be galactosemia; this has been controlled by the feeding of galactose-free milk. The chromosomes of his parents and his all siblings were examined. The father and eldest brother had normal male karyotypes and the mother, two brothers and one sister had the same karyotype as the proband. They had no clinical abnormalities except for dextrocardia in the sister and obesity in the mother and sister. It is very difficult to identify the additional portion of chromosome, but we are investigating along these lines:

(1) Comparison of their clinical findings with those of various chromosomal syndromes; associated with dextrocardia such as 13 trisomy and 10p trisomy.
(2) Study of detailed band patterns made by high-resolution analysis of light and shadow grades (Niihira, Jpn. J. Human Genet.)
(3) Assay of the concentration of enzymes involved in the metabolism of galactose.

C 18

Pseudodicentric18の疑われた一症例．藤沢美朗，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），尾崎陽子，星野道雄（川崎製鉄健康保健組合　千葉病院　小児科）．
A Case of suspected Pseudodicentric 18. Yoshio FUJISAWA, Toshihiro GOTO, Masatoshi MATUDA and Saburo OKUBO (Clin. Lab., Shionogi Co., Ltd.), Yoko OZAKI and Michio HOSHINO (Dept. Pediatr., Kawasaki-Seitetsu Hosp., Chiba)

患児は，在胎39週2日，1,982 g，健康な両親の第3子として出生した女児で，妊娠時，羊水過多が認められた．出生時，早期破水（5時間），切迫仮死，全身チアノーゼ，アプガースコア4点（1分後），同6点（5分後），小顎，耳介低位，耳介変形，後頭部突出，股関節開排制限，筋緊張低下，大陰唇の縮小などを認め，エコー，X線検査の結果，脳梁欠損，脳室拡大，左心房拡大，嚢胞腎などを認めた．生後約2ケ月で，呼吸不全により死亡した．

染色体検査は，末梢血培養法の後，GおよびQ分染法により分析した．その結果，2本の18番染色体が短腕同士で結合した特徴のある派生染色体を認めた．核型　46, XX, ter rea(18;18)(qter→cen→p11::p11→qter)．その切断点はp11であり，サブバンドまでの同定は困難であったが，短腕のほぼ末端付近と考えられた．またC分染法による分析の結果，2つの動原体を認め，かつ一方の動原体は各染色分体に分かれて存在していた．尚，両親の核型は正常であった．

本症例においては，両親がともに正常核型であることより，この派生染色体は突然変異により生じたものである．また短腕末端でrearrangementがあり，短腕末端に微細な欠失が生じていると考えられる．このため通常の18番トリソミーと比較して，臨床像に何らかの相異が生じた可能性がある．

C 19

FOUR DOWN SYNDROME PATIENTS WITH APPARENTLY NORMAL CHROMOSOMES IN A FAMILY. Hiroko KAWASHIMA (Dept. Pediatr., Kanazawa Univ., Kanazawa), Tatsuroh IKEUCHI (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo), Nobuaki OGASAWARA (Dept. Genet., Aichi Pref. Colony, Kasugai), Kazumi IKAWA and Emiko NAKAYAMA (Ishikawa Health Service, Kanazawa)

Four Down syndrome patients were reported in a family. They were three first cousins, two of them were sisters, and their first cousin once removed. The superoxide dismutase (SOD) levels in red blood cells were measured in two sisters and their parents and were within normal range. They had a partial trisomy 21q22.1 qter resulting from parents' translocation, t(?:21)(?:q22.1) which was very difficult to detect without high resolution banding because of no significant changes of band pattern or length of chromosome 21 in G-banded analysis.

We recommend the parents' chromosome tests by high resolution banding when Down syndrome patient has apparently normal chromosomes in both lymphocytes and fibroblasts.

C 20

A CASE OF KLINEFELTER'S SYNDROME ASSOCIATED WITH CEREBRAL ANEURYSM AND VARIX CRURIS. Noriko NAKADA, Hiromu FUNAKI, Eiji HOSHINO, and Kazuyuki ISHITOBI (Dept. Intern. Med., Tottori Univ., Yonago)

The patient, a 23-year-old male, had mental retardation since his childhood. He underwent an surgical operation for varices in the leg causing ulcer. His height was 186.5 cm, his weight 74.5 kg, his arm span 182 cm, with cubitus varus and redioulnar synostosis. The testes were 2.5 by 2 cm. The axillary and pubic hair was sparse. The maxilla and glabella were protrusive like acromegaly. The serum testosterone level was normal. The serum LH and FSH were elevated basally and responded to LH-RH exaggeratedly. The serum growth bormone, basally normal, responded to LH-RH and TRH administration. An aneurysm of the right carotid artery was revealed by angiography. The cytogenetic study of his peripheral lymphocytes showed 48, XXYY.
There are 14 cases of 48,XXYY karyotype reported in Japan, but the association of cerebral aneurysm was seen only in the present case.

C 21

MYOTONIC DYSTROPHY ASSOCIATED WITH 47 XYY SYNDROME. Atsutoshi ASANO,Naoyasu MOTOMURA, Shingo YOKOTA, Hiroshi YONEDA, Toshiaki SAKAI (Dept. Neuropsychiatry, Osaka Medical College, Osaka) and Shigetoshi TSUTSUMI (Minatogawa Hospital,Kobe)

We report here a case of combined autosomal dominant myotonic dystrophy and 47 XYY syndrome. Such a case has never been reported to date. Patient is a 37 year-old, 183 cm tall man with clinical manifestations of mental retardation, abnormal sexual behaviors, cataract, muscle atrophy and myotonia. The chromosomal study revealed that his karyotype is 47 XYY. His sister also has myotonia, but without chromosomal abnormality. There are 6 cases of myotonic dystrophy associated with chromosomal abnormalities reported in literature : 5 cases with Klinefelter syndrome and one with Down syndrome. Although the occurrence of these two disorders in the same patient could have been coincidental, it is also possible that myotonic dystrophy predisposes the patient to a chromosomal aberration.

C 22

UNUSUAL KARYOTYPIC VARIABILITY ASSOCIATED WITH dic(Yq) AND PARTIAL PENTASOMY Yq.

Kazuso IINUMA, Yoshiyuki HIRAISHI, Mizuki KATO, Sachiko YOSHIHARA (Div.Clin.Dysmorph.,Nat.Child.Med.Res.Center, Tokyo) and Nobuko HASHIMOTO (Dept. Endocr. Metabol.,Nat.Child.Hosp.,Tokyo)

Cytogenetic studies on a one-month-old girl with ambiguous genitalia revealed her karyotype to be mos 45,X/ 46,X,dic(Yp11.3). The ratio was 218 to 196, respectively. Furthermore, four cells were 47, XY, dic(Yp11.3), three cells were 47, XYY, and each of other three cells had a karyotype, 46, XY, 47, X,dic(Yp11.3), dic(Yp11.3) and 47, X, dic(Yp11.3), tri(3cen)(Yq), respectively. Ontogenetic explanation for that karyotypic variability was based on the assumption that recombination within a ring structure led to the occurrence of anaphase lag with or without breakage of dic(Yp11.3) at the union site. In one cell with a dic(Yp11.3), there was additionally a triradial rearrangement composed of three Y-chromosomes, though its precise structure could not be available. This is the first evidence for the human cell with partial pentasomy Y. Laparotomy confirmed clinical diagnosis of mixed gonadal dysgenesis and gonadectomy was done.

C 23

STUDY ON STEROID SULFATASE ACTIVITIES IN CELLS OF SEX ANOMALY PATIENTS WITH VARIOUS TYPES OF X-CHROMOSOME ABNORMALITIES.

Kiyomi YAMADA and Mitsuru SHINOHARA (Div.Genetics, Nat.Med.Center Hosp. and Dept.Urology Komagome Hosp., Tokyo)

The steroid sulfatase (STS) gene has been assigned to the terminal portion of the short arm of human X chromosome (band Xp223), and the chromosomal region is thought to be free from the Lyon's X-inactivation phenomenon. We measured STS enzyme activities in cells of normal individuals as well as sex anomaly patients, and studied on the gene dosage effect in the expression of STS enzyme activity. The cellular STS activity was measured by the radioassay method using H^3-dihydroepiandrosterone sulfate as a substrate. The STS activities in normal placental tissues showed a significant difference between males and females, and the ratio of mean values of males and females was 1:1.998 very close to the theoretically expected ratio. In EBV-stimulated lymphoblastoid cells and skin fibroblasts from normal individuals, the sex difference was also clearly demonstrated. From analysis of 13 sex anomaly patients including XXY, XXYY, and XX males, two XX true hermaphrodites, two patients with a X/Y translocation, and a XY female, probably hemizygotes for the STS gene were revealed in two patients; a XX male and a mother with a X/Y translocation.

C 24

A CASE REPORT OF PRENATALLY DIAGNOSED PALLISTER MOSAIC SYNDROME.
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A Japanese woman was referred to our clinics at the 18th week of gestation for her 39 years of age. Routine ultrasound evaluation revealed unusual swelling of the fetus' neck. Because of this abnormality, amniotic-fluid cell culture was attempted and it revealed the presence of an extra metacentric chromosome in addition to a male karyotype in all cells analysed. The pregnancy was terminated in the 19th week and the following findings were noted at autopsy. The fetus weighed 475g. The skull was markedly large and the neck could not be distinguished because of cervical hygroma. He had hydrops fetalis, hydrocephaly, bilateral auricular dysplasia, and bilateral polysyndactyly of the feet. Moreover, cystic dysplasia of the kidney was noted. Blood culture from the fetus revealed a normal karyotype in the majority of the cells studied but in 17% there were cells with an extra metacentric chromosome. His fibroblast culture showed every cell with an extra metacentric chromosome. We concluded the extra chromosome to be the i(12p) because of that G-Banding pattern.

C 25

ENHANCED ACCUMULATION OF HYALURONATE IN THE CULTURE OF SKIN FIBROBLASTS FROM THREE PATIENTS WITH COFFIN-LOWRY SYNDROME. Kiyoshi MIYAZAKI, Tsutomu YAMANAKA (Dept. Pediatr., Cent. Hosp., Aichi Pref. Colony, Kasugai) and Atsuhiko OOHIRA (Dept. Embryol., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Glycosaminoglycans (GAGs) were isolated either from the medium or from the cell layer of cultured skin fibroblasts from three unrelated male patients with Coffin-Lowry syndrome. The hexuronate content in the cultures of Coffin-Lowry fibroblasts was significantly higher than that of normal fibroblasts. Quantitative analysis of GAGs was carried out by measuring optical density at 615 nm of Alcian blue-stained spots on electrophoretograms. Increase in the hyaluronate content was found both in the culture medium (Case-1, 670; Case-2, 601; Case-3, 555; a normal control, 278 μg/μmol DNA) and in the cell layer (Case-1, 64.0; Case-2, 54.4; Case-3, 101.2; a normal control 28.7 μg/μmol DNA) of Coffin-Lowry fibroblasts. In addition , the incorporation of [^{14}C] glucosamine into hyaluronate was similarly activated in skin fibroblasts from patients, suggesting the active biosynthesis of hyaluronate by cultured skin fibroblasts from Coffin-Lowry syndrome.

C 26

DUBOWITZ SYNDROME WITH GROWTH HORMONE DEFICIENCY. Akihiko KODAMA, Kouji SAMESHIMA, Yukihisa MATSUDA, Seigo ONO, Masato KUWAHATA and Kashama TSHIANI (Dept. Pediatr., Kagoshima Univ., Kagoshima)

A 6-year-old boy was referred for evaluation of short stature and facial dysmorphy. He was the first child of two sibs of nonconsanguineous parents. His mother was 27 years old and his father was 33 at the time of conception. The 40 week pregnancy was uncomplicated. Maternal consumption of alcohol was denied. Labor and delivery were uneventful. His birth weight was 2620g.

During the first several months of life he did not feed well, and had repeated otitis media and eczema.

At 1 month of age he weighted 3095g, at 38 months 8670g. He walked and spoke monosyllable words at 22months.

When he was 6 years old his height was 103cm(-2.4 S.D.), weight 12.2Kg(-3.2 S.D.), head circumference 45.3cm(-4.2 S.D.). His clinical features were microcephaly, telecanthus, ptosis-blepharophimosis of left upper lid, retrognathia, bilateral clinodactyly of fifth fingers, mental retardation and hyperactivity.

Hormonal tests showed growth hormone deficiency due to hypothalamic dysfunction.

It is hoped that further investigation on a sufficient number of cases will clarify the relationship between the short stature and growth hormone deficiency in Dubowitz syndrome.

C 27

TERMINAL REARRANGEMENTS AND TRIRADIALS INDUCED BY APHIDICOLIN IN NORMAL HUMAN LYMPHOCYTES. Akira KUWANO and Tadashi KAJII (Dept. Pediat., Yamaguchi Univ. Sch. Med. Ube)

Peripheral blood lymphocytes from two healthy women were cultured for 96 h in MEM, 0.4μM aphidicolin with 0.02% dimethyl-sulfoxide was added 72 h prior to harvest, chromosome preparations were made, and 1,000 metaphases were screened from each women. Together, 61 terminal rearrangements were scored without preferential patterns of association. A total of 92 triradials were observed, with the site 3p14 most frequent among them. Aneuploidy, structural rearrangements and breaks and gaps were frequent among the cells scored.

C 28

DERMATOGLYPHIC FINDINGS OF RATS WITH CHROMOSOME ABNORMALITIES.

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Dermatoglyphic abnormalities are observed frequently in patients with chromosome aberrations, but no similar observations have been made in animals. Recently, dermatoglyphic characteristics were described in the rat. Reciprocal translocations were induced by γ-irradiation. The animals used were obtained from among offspring derived from the original mutant rats, one between chromosomes Y and 11 in strain NIG-III, and the other between chromosomes 1 and 12 in strain WKS. In the present study, palmar dermatoglyphics were examined in 12 rats with 7 different abnormal karyotypes. In a rat with unbalanced karyotype, 43,X,+der(Y),+der(Y),t(Y;11)(q11;p12), showed remarkable distortion of ridge arrangements on the thenar and III interdigital pads. One of the other two rats with unbalanced karyotype, 42,X,der(Y),-11,-11,+der(11),+der(11), presented an unusual extra ridge on one pad. Dermatoglyphics were not unusual in the rats with other 5 abnormal karyotypes, including two balanced translocations. We are continuing the dermatoglyphic survey in offspring derived from the translocation between chromosomes Y and 11.

C 29

INFLUENCE OF QUINOLONE ON THE HUMAN CHROMOSOMES.

Mariko UEHARA, Mitsushiro KIDA (Dept. Pediat., Teikyo Univ., Tokyo)

We analysed chromosomes of the six patients with congenital anomalies and three their parents to study the influence of quinolone (pyridine carboxylic acid) on the human chromosomes. Their karyotypes were; 47, XY,+21; 47,XX,+21; 45,X; 46,XY,t(3;4)(q26;q21); 46,XX; 46,XY. The peripheral lymphocytes cultured during 72 hours were treated for 24 or 72 hours with 1-100 μg/ml of nalidixic acid (NA) or one of the new quinolone (NQ) which is currently under study. The number of chromosomes were decreased by dose dependency from 1 μg/ml during 24 hours. In further higher doses the contraction of chromosomes was increased and mitotic index was decreased SCE(sister chromatid exchange) tended to increase by dose dependency. Especially in the patients with chromosomes t(3;4) SCE increased significantly by treatement with 10 μg/ml NQ during 72h. The number of SCE per cell at break point in t(3;4) were 0.35, 0.36 by 25, 50 μg/ml during 72 h. These data were significantly higher than 0.14 in control. These various chromosomal abnormalities were more frequently observed in NQ than NA.

C 30

MAPPING OF HUMAN GENES USING HUMAN-MOUSE SOMATIC CELL HYBRID CLONE PANEL. Jun KUDOH, Shinsei MINOSHIMA and Nobuyoshi SHIMIZU (Dept. Mol Biol., Keio Univ. Sch. Med., Tokyo)

Recently, we have cloned a 5.2-kb EcoRI fragment of placental DNA which hybridized weakly to the 5'-1kb EcoRI fragment of insulin receptor (INSR) cDNA probe. The restriction map of this genomic fragment revealed that only the 0.3-kb BglII-PvuII fragment is homologous to the INSR probe. Although the complete structure and function of the gene remains to be determined, we designated this gene as INSR2. We perfo-rmed chromosome assignment studies using DNAs from 14 clones of human-mouse cell hybrids by Southern blot hybridization technique. The results indicated that the 5.2-kb EcoRI fragment is present only if these hybrids contained human chromosome 7. The analysis using 4 clones of GMA-series hybrids containing different translocation chromosome 7's provided evidence that the INSR2 gene is localized to the p13-q22 region of chromosome 7. Using the same hybrid panel, human myeloperoxidase (MPO) gene was assigned to chromosome 17 (collaboration with M. Yamada, Inst. Protein Res., Osaka Univ., Osaka). We also mapped genes for phosphoribosyl pyrophosphate (PRPP) synthetase subunits PRSI and PRSII to human chromosome Xq21-qter (designated as PRPS1) and Xpter-q21 (designated as PRPS2), respectively (collaboration with M. Taira and M. Tatibana, Dept. Biochem. Chiba Univ. Sch. Med., Chiba).

C 31

染色体異常と遺伝子増幅（第4報）—同一細胞にみられたdminとHSRの意義—稲澤譲治[1]・阿部達生[1]・井上　清[2]・青木繁明[3]（[1]京府医大・衛生・[2]大阪公衆衛生研究所・[3]京府医大・眼科）. CHROMOSOME ABNORMALITIES AND GENE AMPLIFICATION (IV): OBSERVATION OF dmin AND HSR IN THE SAME METAPHASES IN Y-79. Johji INAZAWA[1], Tatsuo ABE[1], Kiyoshi INOUE[2], Shigeaki AOKI[3] ([1]Dept. Hygiene. Kyoto Pref. Univ. Med., Kyoto ; [2]Osaka Pref. Inst. Public Health., Osaka ; [3]Dept. Ophthalmol. Kyoto Pref. Univ. Med., Kyoto)

腫瘍細胞やその樹立株では，特定の遺伝子増幅を示すdouble minute chromosomes（dmins）やhomogeneously staining region（HSR）の出現が知られているが，両者が同一の細胞で観察されることは，極めて稀とされていた。今回，網膜芽細胞腫樹立株Y－79において，HSRとdminを同時に認め，遺伝子増幅と染色体変化を知る上で興味が持たれたので報告する。型のごとくに染色体標本を作製し132個の細胞を分析した。modeは46にあり，全て1pHSR，4p+，5p−，11p+，12q+，13p+のマーカー染色体を有し，本株がGilbertらの報告するY－79 T－79株に由来することを確かめた。分析細胞中5個（3.8%）に1pHSRと同時に2～4個のdminを認めた。Southern blot法では32倍以上のN－*myc*増幅があり，*in situ* hybridization法を用いた検討の結果，1pHSRでの増幅を確認した。*in vivo*, *in vitro* cultureで，株細胞にみるHSRとdminは互いに移行し得る染色体変化とされている。さらにdminの出現機序の一つにHSRのbreak downやexcisionが考えられている。しかし，今回1pHSRに加えて同時にdminが観察されたことより，本細胞ではこの機序によらず，新にdminが出現したものと考えられる。

C 32

CYTOGENETIC CHARACTERIZATION OF CELL LINES ESTABLISHED FROM HUMAN RENAL CELL CARCINOMA. Mitsuaki A. YOSHIDA, Tatsuro IKEUCHI, Akira TONOMURA, (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo), Masami WAKISAKA, Jun SHIMAZAKI (Dept. Urol., Chiba Univ., Chiba) and Yuichi TACHIBANA (Dept. Urol., Tokyo Med. & Dent. Univ., Tokyo)

Four parmanent cell lines were successfully established in vitro from human nonfamilial renal cell carcinomas. Tumor cells were disaggregated from primary tissues by treatment with collagenase II and cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum. Modal chromosome number was near-diploid in two cell lines (RCC 826 and RCC 23), near-triploid in one line (RCC 31) and near-tetraploid in the remaining one cell line (RCC 19). Detailed analyses of Q-banded chromosomes demonstrated various structural changes involving chromosomes #1, 3, 6, 8, 9, 14, 17 and 19. The chromosome #3p abnormalities, which have been observed in the previous studies as characteristic changes in renal cell carcinoma, were also found in all the cell lines. They include a translocation between #3p and #8q in both RCC 826 and RCC 23, a partial deletion of #3p in RCC 19 and two translocations between #3p and unidentified chromosome segments in RCC 31. These clonal rearrangements were stably maintained in each cell line under the prolonged culture condition. The cell lines with the #3p structural changes may be valuable for further studies especially based on a molecular level.

C 33

GENETIC COUNSELING AND ITS FOLLOWUP STUDY (Ⅳ) Masao NAKANAGA, Yoshiyuki OHNISHI, Akira TOKUDA, Shigeaki NAKAZAKI, Tatsuro MUTOH, Motozumi NOMURA, Mikio HIRAYAMA, Norio FUJIKI (Dept. Int. Med., Fukui Med. Sch., Fukui) and Kazuo MANO (Nagoya First Red Cross Hosp., Nagoya)

There have accumulated 2287 cases of genetic counseling in Kyoto, Aichi and Fukui areas for the past 27 years.

Significantly more persons came to the counseling unit through local health authorities in Fukui than in other areas, probably due to recent activities of public health nurse. The most common motive was the recurrence risk estimation of the disease, at the time of marriage (39 %) and of pregnancy (27 %), and the contents were occupied by polygenic diseases including malformation and constitution (34 %), hereditary diseases (27 %), and consanguinity (17 %) etc.

Followup study in Kyoto on 1972 for 370 cases counseled during 1969 - 1975, was successed on 81 families (22 %) and majority of families seeking advice accepted our advices and acted accordingly. Following same studies on 1977 and 1984 in Aichi and Fukui respectively, the response rates were 46 % and 42 %, due to immediate asking and it was evident that the genetic prejudices have still existed but common sense was spread gradually in general public correctly.

C 34

The changes of the genetic markers after transplantation of bone marrows

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The changes of genetic markers of blood or saliva were investigated in three donor-host pairs in bone marrow (BM) transplantation. The blood and saliva were collected from both of donors and hosts before and after the transplantation. The results had resembled in the three pairs. Some of the genetic markers in hosts were different from those in donors. These genetic markers split up two groups from their response after BM transplantation. Group 1: The genetic markers of host and changed into donor type after BM transplantation, which were ABO, MN, P, Ph, Kidd, EsD, PGM, Acp system. Group 2: The genetic markers of host had not changed into donor type even after BM transplantation, which were Lewis, Se, Hp, Tf, Pa, Pr, PmF, Db system. Other genetic markers, Lutheran, Kell-Celano, Diego, Fuffy, Xg, Gc, Gm, Km, Complimental system, GLO, 6PGD, Pb, PIF, Amy1 system and Leucocyte type (A, B,C,DR,DQ), were same in type between hosts and donors. These finding of the genetic markers gave us many informations on the reproduction and the disappearance of the genetic substance.

C 35

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

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The study was carried out to examine the comparison of the effects of counterstaining with methyl green,crystal violet and malachit green as non-fluorescent dyes on di-9-acridylphenylhydrazone as acridine or bis(2- quinolyl)-9,10-anthrylhydrazone as quinoline derivative fluorescence of human chromosomes.The pairing of di-9-acridylphenylhydrazone with non-fluorescent A-T specific DNA dye methyl green and crystal violet produced greatly enhanced contrast of paracentromeric regions. These regions were the paracentromere of chromosome 1,9. But the regions counterstained with malachit green did not produce the enhanced contrast.These findings were similar to the results obtained from the counterstaining with the three non-fluorescent dyes on bis(2-quinolyl)-9,10-anthrylhydrazone.