日本人類遺伝学会第 30 回大会一般講演要旨

Abstracts of General Contribution, the 30th Annual Meeting of the Japan Society of Human Genetics

I-1. A Case of Deletion 2q: **Hidetsugu MIZUUCHI, Noriyoshi KASA** (Dept. Pediatr., Natl. Okayama Hosp., Okayama) **and Ryozo KASAI** (Asahigawa Jidoin Child. Hosp., Okayama)

The patient was a male newborn who was the second child of unrelated and healthy parents. His mother was 28 years old and his father 31. His only sibling was a 2 year old healthy boy. His birth weight was 2,650 g. Apgar score was 5 at one minute. He was admitted because of mild asphyxia and multiple deformities. The following deformities were observed: wide prominent forehead, small face, microstomia, malformed ear, low-set ears, webbed neck, low-set nipples, syndactyly of the 2nd and 3rd fingers in both bands and the 2nd to 4th toes in both feet, and bilateral simian creases. At the age of 4 days heart murmur was found and ventricular septal defect was suspected by echocardiogram. CT of the head showed mild ventricular dilatation. In the high resolution banding studies in peripheral leucocytes, the karyotype of the child was 46,XY,del(2)(q37.1). The chromosomes of both parents were normal. Erythrocyte IDH1 (isocitrate dehydrogenase) activity, measured by the method of Bergmeyer and Bernt (1974), was normal, indicating that the IDH1 locus can be excluded from the deleted region in the present case.

I-2. Interstitial Deletion of the Long Arm of Chromosome 3 : del(3) (q12q21) *de novo*: **Shizuhiro NIIHIRA, Hiroko FUJITA** (Dept. Child Health, Osaka City Univ., Osaka), **Tuneo HARUMOTO** (Dept. Pediatr., Mimihara General Hosp., Osaka), **Hirokazu SUZUKI and Hideo MATSUMOTO** (Dept. Legal Med., Osaka Med. Coll., Osaka)

We report clinical findings and gene mapping investigation in a boy with a *de novo* interstitial deletion of a segment q12→q21 of chromosome 3, del(3)(pter→q12::q21→qter). He was born primipara with cesarean section at 40 weeks, because of the opacity of the amniotic fluid. Birth weight was 3,140 g, body length 48.5 cm, and head circumference 35 cm. His father was 30 years old and his mother 29 years old. Physical examination presented minor anomalies such as flat occiput, asymmetric ears with the right low set and left hypoplasia of auricle, redundant skin of neck, umbilical hernia, small

penis, and simian crease in the left hand. Routine X-p, CT, and other routine laboratory and biochemical examinations were all within normal limits. He could not sit head steady and could not follow moving objects sufficiently at one year of age. Thirty-six kinds of blood type were investigated in the boy and his parents. The results gave no information as to their gene location except for negative data on Tf, C6 and FXIIIB. Incidentally, the boy and his father were found to have a rare variant of B-locus product of C4 allotype. The gene URIC is provisionally assigned to 3cen→q21 region (Human Gene Mapping 7, 1984), but orotic acid in our patient's urine was within normal limits. To our knowledge, 3 cases of long arm deletion of chromosome 3, del(3)(q23q26), have been described. The deleted regions in the previous cases and in our case are not overlapping each other.

I-3. 母親の均衡転座 t(4p−;10q+) に由来する 4p トリソミーの同胞例：鮫島幸二，児玉昭彦，チャニ・カシャマ（鹿児島大・医・小児）. **Two Siblings with Trisomy 4p due to Maternal t(4p−;10q+) Balanced Translocation:** K. SAMESHIMA, A. KODAMA and K. TSHIANI (Dept. Pediatr., Kagoshima Univ., Kagoshima)

4p トリソミーは，そのほとんどが親の平衡転座によることが知られている．今回われわれも，母親の平衡転座に由来する 4p トリソミーの同胞例を経験したので報告する．症例 1 は 7 歳男児．在胎 40 週，2,600 g で出生．生後 3 カ月目に体重増加不良と手掌横線，第 5 指内湾などの精査目的で来院．染色体検査の結果，母親に平衡転座 46,XX,t(4;10)(p14;q26) を認めたため，患児は核型 46,XY,der(10),rcp(4;10)(p14;q26)mat の 4p トリソミーと診断された．その後 3 歳時に全身性間代謝性痙攣が出現し，てんかんの診断で現在も加療中である．7 歳時の DQ は 43 であった．

症例 2 は，症例 1 の弟で，妊娠 20 週目に羊水検査をうけているが正常といわれていた．在胎 39 週，1,794 g で出生．生後 5 カ月目に保健所にて心雑音を指摘され，精査のため当科受診．顔貌が兄と類似し，手掌横線や内反足などから染色体を検査したところ，兄と同様の 46,XY,der(10),rcp(4;10)(p14;q26)mat を認めた．1 歳時の DQ は 65 であった．4p を含む親の平衡転座から 4p+ や 4p− が生ずる場合はほとんどが隣接 I 型分離で，それぞれ 25% の危険率であるが，4p+ の同胞内頻度をまとめてみると 4p+ は 50%，4p− は 3% 程度であった．

I-4. Proximal 6q Monosomy: Yoshifumi YAMAMOTO, Noriko OKAMOTO, Hirohiko SHIRAISHI and Masayoshi YANAGISAWA (Dept. Pediatr., Jichi Med. Sch., Tochigi)

Deletions of 6q are exceedingly rare and the previously reported cases have involved different monosomic regions. McNeal *et al.* (1977) and Young *et al.* (1985) described patients with proximal 6q monosomy, del(6)(q13q15), showing variable multiple congenital malformations. We reported a 13-year-old boy who had an interstitial deletion of the long arm of chromosome No. 6: 46,XY,del(6)(pter→q13::q15→qter). A peculiar facial

appearance or multiple congenital anomalies including facial asymmetry, vertebral anomalies, heel valgus with flat feet and congenital heart disease may form a part of the characteristic features of proximal 6q monosomy.

I-5. 父親の均衡転座 46,XY,t(7;13)(q32;q34) に由来する holoprosencephaly をもった monosomy 7q の一症例：笹本喜代・高林俊文・小沢信義・曽宗仁・佐々木裕之・佐藤真澄・佐藤 章・矢嶋 聰（東北大・医・産婦人）．**A Case of Holoprosencephaly with a Terminal 7q Deletion Due to a Paternal Transloation t(7;13)(q32;q34):** K. SASAMOTO, T. TAKABAYASHI, N. OZAWA, S. SOU, H. SASAKI, M. SATO, A. SATO and A. YAJIMA (Dept. Obstet. Gynec., Tohoku Univ. Sch. Med., Sendai)

第 7 番の染色体部分欠損症の報告は，本邦においては数例にすぎない．今回，われわれは妊娠 24 週時，超音波断層法により致死的多発奇形を診断し，早産させ，染色体分析を施行した結果 monosomy 7q であった症例を経験したので報告する．母親は 23 歳 0 妊 0 産で，妊娠 24 週にて胎児異常が疑われて当科紹介となる．

当科での超音波断層所見では BPD 59mm，単脳室，brain mantle はほとんどなく，thalamus は左右が正中で癒合しており，前後に長い洋梨型を呈していた．第 3 脳室は認められ，cerebellum はおおよそ正常であった．これらは holoprosencephaly の所見に一致し，alobar type の holoprosencephaly ではないかと考えられた．顔面は両眼ともに明らかに描出されず，眼球の異常が考えられた．カウンセリングの後に早産術を施行した．体重 748 g，身長 33 cm の女子であった．外表奇形としては，分娩前の超音波断層所見とほぼ同じであった．児の血液を 72 時間培養後染色体標本を作製し，G-分染法を行った結果，No. 7 染色体長腕の部分欠損（切断点 q32）が認められた．その後の両親の染色体検査にて，父親が t(7;13)(q32;q34) の転座保因者であることが判明したので，患児の核型は 46,XY,−7,+der(7) (7;13)(q32;q34)pat と確認された．

I-6. A Case of 11q Distal Monosomy: Yuko ENDO, Akihiko YABUHARA and Taro AKABANE (Dept. Pediatr., Shinshu Univ., Matsumoto)

A girl was born to non-consanguineous parents. Her father was 33 years of age and her mother 35. Her sister was 1 year of age and healthy. One spontaneous abortion was observed before her. After 42 weeks gestation, her birth weight was 3,440 g and body length was 51.0 cm. The pregnancy and delivery course were uneventful. She visited our hospital because of poor weight gain and was hospitalized at 1 month of age when heart murmur and slight anemia were noticed. She showed trigonocephaly, ptosis of eyelids, long eye lashes, bulbous nose, anteverted nostrils, carp-shaped mouth, retrognathia, small nipples, accessory mamillas and sacral dimple. The external genitalia was normal. Her heart murmur turned out to be atrial septal defect with pulmonary hypertension and she was operated 2 years after. She is now 3 years of age, showing growth retardation

and developmental delay especially in speech, though her hearing tests were normal. Her developmental quotient was 69. Dermatoglyphic findings were within normal limits except for clinodactyly and shortness of both 5th fingers. Total ridge count was 159. Chromosome analysis of peripheral blood using G- and R-banding methods revealed a terminal deletion of the long arm of chromosome 11. Her karyotype was 46,XX,del(11)(q23). Double minute chromosomes were also observed.

I-7. Distal Trisomy 14q Syndrome: **Y. WAKITA, K. NARAHARA, Y. TAKAHASHI, K. KIKKAWA, S. KIMURA, H. KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama), **K. EGUCHI** (Dept. Obstet. Gynec., Okayama Univ., Okayama), **H. NOMURA** (Dept. Pediatr. Fukuyama Citizen's Hosp., Fukuyama) **and T. OHARA** (Dept. Pediatr., Fukuyama Natl. Hosp., Fukuyama)

Distal trisomy 14q is less common than proximal trisomy 14q. It has not been established whether distal trisomy 14q is associated with a well-defined syndrome. We described two related cases of distal 14q trisomy resulting from a maternal translocation. Case 1 was a male newborn delivered after 35 weeks gestation, and his birth weight was 1,495 g. He showed facial asymmetry, frontal bossing, hypertelorism, narrow left palpebral fissure, right cloudy cornea, prominent nose, cupid bow lip, micrognathia, posteriorly rotated ears, atresia of external ear canal, overlapping fingers and fifth finger clinodactyly, prominent calcaneus, severe hypotonia. He died of septicemia at one month. Case 2 was a male fetus aborted at 20 week's gestation after amniocentesis. He showed the clinical manifestations similar to case 1, but had no cloudy cornea or atresia of external ear canal. The karyotype of the both cases was 46,XY,-5,+der(5),t(5;14)(p15.3;q31.2)mat. Our cases and seventeen reported cases of distal trisomy 14q in the literature suggest a clinical syndrome, of which specific findings include facial asymmetry, prominent and anteverted nostril, cupid bow upper lip and posteriorly rotated ears. Our cases indicate that the duplication of the segment 14q31$\rightarrow$qter is enough to cause distal trisomy 14q syndrome.

I-8. A Case of Partial Trisomy 17q: 46,XX,inv dup(17) (9q24$\rightarrow$q25.3): **Toshiaki SHIMIZU** (Dept. Pediatr., Juntendo Univ., Tokyo), **Satoru OHBA, Hideki MIYAGUCHI, Tadashi AKIYAMA, Takashi SHIBATA** (Dept. Neonat., Juntendo Izunagaoka Hosp., Shizuoka), **Tamiko SHINOHARA** (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent., Tokyo) **and Tatsuro IKEUCHI** (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

The proposita was born at 38 weeks' gestation to healthy and unrelated parents. Her mother was 29 years old and the father 36 years old when the patient was born. Her

mother has had a previous pregnancy which resulted in spontaneous abortion at 3 months' gestation. Her birth weight was 2,797 g and her length 45 cm. She was transferred to the Juntendo Izunagaoka Hospital shortly after birth, because of her peculiar facies. At admission, the patient showed multiple phenotypic anomalies: narrow palpebral fissures, low-set and malformed ears, thin lips and downturned corners of the mouth, high arched palate, webbed neck, pectus carinatum, small palms and feet, and hirsutism of the whole body. At the age of 10 months, her mental and physical developments were severely retarded. Chromosome analyses using a trypsin-Giemsa banding technique were performed on cultured lymphocytes and skin fibroblasts, and an extra material on the long arm of chromosome 17 was found in all the cells examined. High-resolution banding analysis after ethidium bromide pretreatment identified the 17q+ chromosome as an inverted duplication of the q24.5→q25.3 region. The parents' karyotypes were normal. In the literature, there have been only 6 cases of partial 17q trisomy. Five of these were resulted from a parental translocation, and the remaining one was a *de novo* case. The overall clinical features of our case were very similar to those of the previously reported cases.

I-9. Absence of an Interstitial Deletion in the Short Arm of Chromosome 20 in Patients with Multiple Endocrine Neoplasia Type 2: **Tatsuro IKEUCHI** (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo), **Shin-ichi TAKAI,[1] Tetsuro MIKI,[2] Hideo TATEISHI,[1] Isamu NISHISHO[1]** ([1]2nd Dept. Surg., [2]Dept. Med. Geriat., Osaka Univ. Sch. Med., Osaka) **and Ikuko KONDO** (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Multiple endocrine neoplasia type 2 (MEN-2) is an autosomal dominant inherited disorder characterized by medullary thyroid carcinoma and pheochromocytoma, but the essential nature of the mutation that leads to the development of tumors has remained unknown. Recently Van Dyke and his group demonstrated a minute interstitial deletion within band p12.2 of chromosome 20 in patients with MEN-2 (types 2A and 2B) (*Proc. NAS* **81**: 2525, 1984). To evaluate their interesting finding, we performed high-resolution G-banding by application of ethidium bromide method to lymphocyte cultures from 7 unrelated patients with MEN-2A and 4 control subjects. Well-delineated mitoses at 850–1,000 band stages were selected, in which at least one of the two no. 20 homologues showed a subtle band of p12.2. More than 10 such cells were analyzed in each case. It was found that in all the cases studied the band p12 on both the no. 20 homologues were clearly divided into 2 positive subbands, p12.1 and p12.3, indicating that there was no detectable deletion within band p12.2. Though there was a minor fraction of cells where the band p12.2 was identified only in one of the 2 homologues, the rates of such cells in patients did not exceed those (approximately 10%) in control subjects. In conclusion, the finding by

Van Dyke's group was not confirmed in the present study; we therefore refrain from assigning the MEN-2 locus to the chromosome 20p. The present finding is also supported by the negative linkage data obtained by using an RFLP marker (D20S5) known to be localized to 20p12 (Goodfellow *et al.*, 1984; Miki *et al.*, this conference, SIII-4, 1985).

I-10. 45,X/46,X,dic Yq Mosaicism in a Patient with Turner's Syndrome: **Nakamichi SAITO** (Dept. Obstet. Gynec., Kyushu Rosai Hosp., Kita-Kyushu), **Keiji KATO,[1] Genichi NAKAMURA,[1] Toshio HIRAKAWA[2]** ([1]Dept. Obstet. Gynec., [2]2nd Dept. Pathol., Kyushu Univ., Fukuoka), **Shinichi SONTA** (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai) **and Hiromi SAKAMOTO** (Dept. Genet., Hyogo Coll. Med., Nishinomiya)

Twenty-three cases of dicentric Yq chromosome have been reported in the literature and most of the cases were mosaic, predominantly with a 45,X cell line. In these cases, there was a wide range of variation in phenotype including external genitalia and histology. The present case was a 27-year-old girl who showed primary amenorrhea and some of the Turner's stigmata. Duplication of the long arm of Y chromosome was demonstrated by its typical fluorescent banding pattern and an identification of the dicentric Yq was made by C-banding technique. HY-A was negative. Endocrine profiles suggested the dysgenetic gonads which produced a small amount of Δ^4 androstinedione. Histological examination of extirpated gonads revealed the scattered hilus cells in the stroma and no testicular elements. Gonadal Y-chromatin was negative and no malignant components were found in gonads. The mechanism of the mosaicism in the presence of dic Yq was speculated by Ying *et al.*: breakage in the short arm of the Y chromosome in meiosis I in the father and reunion of the proximal broken ends of the chromatids followed by formation of dicentric chromosome after meiosis II, and centromeric division which is liable to produce 45,X cell lines as a result of the loss of this unstable chromosome in postzygotic mitosis. Review of the literature including our case suggests no paternal age effect on the formation of the dic Yq. The phenotype and karyotype correlation was thought to be proportional to the presence of 45,X cell lines in the various tissues. The present case supports the presence of the testis-determining gene in the pericentromeric region of Y chromosome.

I-11. Psychological Characteristics of Children with Sex Chromosomal Abnormalities: from the Viewpoint of Development: **H. KAWAI** (Psychi. Res. Inst. Tokyo, Tokyo), **M. HIGURASHI, T. TAKESHITA** (Dept. Health Sci., Yamanashi Med. Coll., Yamanashi), **S. EGI, F. TANAKA** (Dept. Pediatr., Univ. Tokyo, Tokyo), **M. SE-**

GAWA (Segawa Child Neurol. Clin., Tokyo) **and S. KATO** (Dept. Pediatr., Yamanashi Med. Coll., Yamanashi)

We reported last year that low mental activity, withdrawn and passive mental attitude, poor emotional expression, and lacks of undulation of emotion were observed in cases with Turner syndrome. The purposes of the present study were to confirm these findings, to study whether some developmental changes in psychological characteristics occurred, and to get cues for psychological and social supports for the syndrome. We readministered Rorschach Test to the same subjects who were examined previously. The results were as follows. Withdrawn and passive mental attitude, poor emotional expression, and lacks of undulations did not change basically, but the following changes were observed: 1) increase in mental activity and productivity, 2) increase in reality testing ability, 3) increase in ability of grasping the world whole and constructively, 4) increase in biological energy and activity, 5) increase in interests, 6) change of the way of experiencing the internal and external world, and 7) increase in emotional response to external emotional stimulation. In order to reveal the reason of these psychological characteristics and changes of these characteristics, it is necessary to analyze these psychological characteristics from the biological viewpoint and to study the patient longitudinally from infanthood. Especially, the characteristics of rearing should be studied. It seems to us that these psychological characteristics and the changes of the characteristics were influenced by psychological and social factors.

I-12. A Case of 46,XY Pure Gonadal Dysgenesis with Mixed Germ Cell Tumor: **Hideki TERAMOTO, Kazushi NOMURA, Masaaki TAKENAKA, Katsunori UEDA, Atsushi FUJIWARA** (Dept. Obstet. Gynec., Hiroshima Univ., Hiroshima), **Kozo OHAMA** (Dept. Obstet. Gynec., Kure Natl. Hosp., Kure) **and Hiromi SAKAMOTO** (Dept. Genet., Hyogo Coll. Med., Nishinomiya)

A 19-year-old phenotypic female was referred for evaluation of primary amenorrhea and lower abdominal pain. The patient had undergone the resection of right gonadal tumor at the age of eight and the pathological diagnosis of the tumor was dysgerminoma. Physical examinations disclosed no external anomaly, poor breast development and well pubic hair development. Gynecological examinations disclosed normal external genitalia and vagina, hypoplastic uterine body and the first-sized lower abdominal mass. Serum FSH and LH levels were elevated, serum estradiol and progesterone levels were low and serum testosterone level was within female normal range. Serum beta-HCG and alpha-fetoprotein levels were elevated. The patient underwent laparotomy, which disclosed the left adnexal mass, normal uterine body and bilateral fallopian tubes. Pathological diagnosis of the left gonadal tumor was mixed germ cell tumor (dysgerminoma, teratoma and endo-

dermal sinus tumor). The patient's karyotype was 46,XY and H-Y antigen was positive. Chromosomal analyses of the tumor revealed that chromosome numbers ranged from 45 to 48 with sex chromosome constitution of XY and 13 monosomy was always recognized in the analyzed cells. The tumor was heterozygous for chromosome heteromorphisms which were identical to those of the patient. Therefore, it was assumed that the tumor had originated from a premeiotic cell or by the failure of meiosis I and the karyotype of the original germ cell had been 45,XY,−13.

I-13. Two Female Cases with Duchenne Muscular Dystrophy Having X/Autosome Translocations: **Fumiko SAITO, Akira TONOMURA** (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo), **Seiji KIMURA** (Dept. Pediatr., Yokohama City Univ., Yokohama), **Nobuko MISUGI** (Dept. Orthoped., Kanagawa Child Med. Cent., Yokohama), **Hideaki TOMI and Hideo SUGITA** (Div. Neuromuscl. Res., Natl. Cent. Nerv. Ment. Muscl. Dis., Tokyo)

Duchenne muscular dystrophy (DMD) is a X-linked recessive disorder affecting mostly males. Recently, ten female cases of DMD with various X/autosome translocations have been reported. In the present study, we found two additional cases of DMD with translocations in 3-year-old (case 1) and 11-year-old (case 2) females. These patients were diagnosed as DMD, on the basis of clinical histories and courses, creatine phosphokinase (CK) levels and muscle biopsy histologies or electromyogram. Since they had negative family backgrounds of DMD and their mothers had normal levels of CK activity, there was no compelling evidences to suggest that the mothers were heterozygous for the DMD gene. Karyotypes of the patients showed 46,X,t(X;4)(p21;q26) in case 1 and 46,X,t(X;5)(p21;q13) in case 2. The exchange point located at p21 on the X chromosome in both cases was very similar to that of previously reported cases. On the other hand, the late replicating chromosome was found to be the normal X in all informative 104 cells (case 1) and 70 cells (case 2) using the BrdU-Hoechst 33258/Giemsa staining technique. As a result, the phenotypic expression of the DMD disease in these cases is thought to be brought about by the translocation which produces the abnormal DMD gene, followed by the nonrandom inactivation of the normal X chromosome.

I-14. Measurement of Cerebellum and Pons with the Cranial Computed Tomography in Chromosome Abnormalities: **Atsushi IESHIMA, Sachio TAKASHIMA and Kenzo TAKESHITA** (Div. Child Neurol., Tottori Univ. Sch. Med., Yonago)

We studied on the growth of the cerebellar and pontine sizes on the morphometric CT examination of patients with chromosome abnormalities. They included 54 patients with

Down syndrome, 7 with 5p− syndrome, 5 with trisomy 18, 20 with other autosomal abnormalities (trisomy or monosomy), 4 with sex chromosome abnormalities, and 4 with balanced rearrangement. Normal controls used were 138 subjects aged from 0 to 29 years. The lateral and sagittal sizes of both the cerebellum and the pons were measured directly on Polaroid prints or X-ray films and then calculated into actual values by CT reduction rate. The cerebellar and pontine sizes in Down syndrome showed no differences from those in control group until 5 months of age, but showed significantly small values compared with those of controls after 6 months. All of seven CT scannings of 5p− syndrome displayed small cerebellum, small pons and dilated lateral ventricles from 0 month to 18 years. Five CT scannings of trisomy 18 under 2 months of age showed cerebellar hypoplasia (small cerebellum and dilated ciserna magna) and relatively small pons. In most cases of other autosomal abnormalities, the sizes of cerebellum and pons were small, whereas those were within normal range in balanced rearrangement or sex chromosome abnormalities. Normal growth of pons and cerebellum until 5 months in Down syndrome may suggest effects of intrauterine maternal factors.

I-15. Studies on the Length Variation of the Human Y Chromosome in Normal and Patient Populations: **Kiyomi YAMADA** (Div. Genet., Clin. Res. Inst., Natl. Med. Cent. Hosp., Tokyo)

To explore biological and clinical significances of Yq heterochromatin in man which varys quantitatively among individuals, we have been studying the relation of Y chromosome length to the height of individual, chromosomal non-disjuction, male sterility, and the fetal loss. So far we have accumulated data from length measurements of the Y chromosome performed in normal as well as 3 different patient populations together with information on the age and the height of individuals. In this communication, we report the present results. Relative lengths of the Yq, Yq11, and Yq12 band stained by QM were obtained by the way that each length was divided by the mean length of 21q's in the same cell, and means were calculated from measurements in five cells per individual. Mean relative length of the Yq were 1.58 in normal males (n=250, ages 20–40 yrs.), 1.57 in azoospermia patients (n=74, karyotype 46,XY), 1.55 in Klinefelter patients (n=114, karyotype 47,XXY), and 1.53 in Down patients (n=200, karyotype 47,XY,+21). Correlation coefficients (r's) between the Yq12 length and the height of individuals were 0.192 in normal males (n=250), 0.283 in Klinefelter patients (n=53, ages 17–45 yrs.), and 0.128 in azoospermia patients (n=46, ages 17–40 yrs.). Statistical analysis revealed that the mean length of the Yq in Down syndrome was significantly shorter than that of normal males ($p<0.01$). This deviation may be related to natural selection among 21-trisomic fetuses before birth.

The correlation of Yq heterochromatin with height proposed by Yamada *et al.* (*Hum. Genet.* 1981) seemed to be further substantiated by the present study.

I-16. 精神疾患の細胞遺伝学的研究（第 1 報）：辻　敬・豊田勝弘・米田　博・木戸上洋一・堺　俊明（大阪医大・精神）. **A Cytogenetic Study on Mental Illness:** Takashi TSUJI, Katsuhiro TOYODA, Hiroshi YONEDA, Yoichi KIDOGAMI and Toshiaki SAKAI (Dept. Psychiat., Osaka Med. Coll., Takatsuki)

一般に，性染色体異常は常染色体異常に比べ身体的特徴は著明でなく，その発見は困難であるが，精神医学的にはさまざまな精神症状や社会的逸脱行動を呈し注目されている．今回，精神疾患と性染色体異常との関連を明らかにする目的で，13 の精神病院に精神分裂病，躁うつ病，てんかん，脳器質性精神障害，中毒性精神障害，精神発達遅滞等の診断で入院している男子 1,993 名，女子 1,479 名，合計 3,472 名に対してスクリーニングを行った．その結果，男子ではXクロマチン陽性は 7 名 (0.35%) で，そのうち 5 名 (0.25%) が 47,XXY であり，残り 2 名は 46,XY である．ダブルYクロマチンは 4 名 (0.20%) で，そのいずれもが 47,XYY である．Yクロマチン陰性は 1 名 (0.07%)で，46,XYq− である．女子では，ダブルXクロマチンは5名(0.34%) で，そのうち 4 名 (0.27%) が 47,XXX であり，残り 1 名は 45,X/46,XX/47,XXX の核型である．Xクロマチン陰性は 1 名で，その核型は現在培養中である．この結果を新生児集団における性染色体異常の頻度と比較すると，XXY と XXX では統計学的に有意差が認められるが，XYY では出現率は高いが有意差はない．いま，全症例のうちより精神分裂病に限定すると，この傾向はさらに強くなる．しかし，各精神病院での診断基準が一致していないことや，母集団が小さいことなどもあり，今後さらに症例を増やして検討していく必要がある．

I-17. Selective Elimination of Chromosomally Unbalanced Sperm. Studies with an Experimental Animal: Shin-ichi SONTA (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai) **and Hiroshi KASEKI** (Dept. Obstet. Gynec., Nagoya Univ., Nagoya)

Selective elimination of chromosomally unbalanced gametes, especially sperms, is presumed to be one of the causes of the inclination of chromosome abnormalities which are seen in spontaneous abortion and newborns. In man, however, it is difficult to observe directly chromosomes of the spermatocytes and sperms. Using Chinese hamsters heterozygous for various reciprocal translocations [T(2;10)3Idr, T(1;3)8Idr and T(1;2)9Idr], we studied the relationship between the chromosome constitution and selection of the sperms. In males heterozygous for these translocations, the frequency of meiotic metaphase II (MII) cells with unbalanced chromosome constitution ranged from 54.0% to 60.4%. Compared the ratio of chromosome constitution in one-cell embryos from the backcross of male translocation heterozygotes with the expected ratio from the MII counts in males, the possibility of the sperm selection by chromosome abnormalities was ascertained. The

results indicated that there was a significant decrease of embryos partially monosomic for chromosome 1 compared with the number of embryos expected from MII scoring. Thus, the sperms bearing such unbalanced chromosomes are possibly eliminated during the period from the second meiotic division to fertilization.

I-18. Origin of *de novo* Chromosome Rearrangements: Satoshi ISHIKIRIYAMA (Dept. Pediatr., Hokkaido Univ. Sch. Med., Sapporo), **Tsutomu KAMEI and Norio NIIKAWA** (Nagasaki Univ. Sch. Med., Nagasaki)

We studied the origin of *de novo* chromosomal rearrengments in six cases, all of which had an abnormality including acrocentric chromosomes. The subjects consisted of two patients with a r(15) and each one patient with 13p+, 14p+, t(14;21), and t(21;21). Peripheral lymphocytes were cultured, and chromosome slides were made by ordinary methods. The parental origin was identified according to the fluorescent heteromorphism using sequential QFQ- and RFA-techniques. We could not determine the origins in three cases with 13p+, r(15), and 5(21;21). These arrangements involve the short arms, and thus the almost all regions which might express, fluorescent heteromorphism were lost, making it difficult to determine the origins. We could clarify the origin in the other three cases: the 14p+ and r(15) case were paternal and the t(14;21) was maternal in origin. These data agree with those of previous studies, which indicated that Robertsonian translocation was predominantly maternal in origin, and the other rearrengements predominantly paternal.

I-19. Spontaneous Incidence of Chromosome Aberrations and Radiosensitivity in Human Spermatozoa (2nd Report): Yujiro KAMIGUCHI and Kazuya MIKAMO (Dept. Biol. Sci., Asahikawa Med. Coll., Asahikawa)

Recently, we established an improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova. Using this method, we analyzed 326 (control) and 822 spermatozoa (irradiated) in addition to our previous data, making a total of 1,417 and 1,978 spermatozoa, respectively. The results were as follows: (1) The spontaneous incidence of human spermatozoa with chromosome aberrations (13.9%) was far higher than those of mouse spermatozoa (1.5%) and Chinese hamster spermatozoa (2.1%). Incidences of aneuploidy and structural anomaly were 1.2% and 12.7%, respectively. The latter included breaks (49.4%), fragments (29.4%), exchanges (20.6%) and deletions (0.6%). The incidence of structural anomaly varied considerably among the 7 donors, ranging from 7.3% to 19.8%. (2) Increases in rates of chromosomally abnormal spermatozoa after X-irradiation were 8.0±4.6% (25 rad), 17.0±4.8% (50 rad), 34.4±3.8% (100 rad) and

67.3±4.4% (200 rad), showing a linear increase of affected spermatozoa. We estimated the contribution of natural radiation to spontaneously occurring structural chromosome aberrations to be 0.02–0.03%. In other words, more than 99.9% of these aberrations are caused by some other factors, because of the following reasons. a) Doubling dose for induction of structural aberrations was calculated to be 38 to 67 rad. b) Many of the aberrations were breaks and fragments. These aberrations were considered to take place during a postmeiotic period from the spermatid stage to ejaculation (about 40 days or more). c) Radiation from natural sources is approximately 10 mm rem during the same duration.

I-20. The Fragile X Syndrome in a Japanese Population of Institutionalized Mentally Retarded Males: **Tadao ARINAMI, Setsuko TAKANAWA** (Ibaraki Pref. Colony Hosp., Ibaraki) **and Ikuko KONDO** (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

A cytogenetic survey for fragile (X)(q27) was performed on mentally retarded males of a mental retardation institution in Ibaraki Prefecture which is located in central Japan. In this survey, we excluded the male patients with constitutional chromosome abnormalities other than the fragile X chromosome. The ages of the patients examined ranged from 6 to 69 years old. Among 243 cases from 236 families, thirteen cases (5.3%) from nine families (3.8%) were detected to have the fragile X chromosome, in percentages of their cells ranging from 3% to 61%. All cases with the fragile X chromosome had been diagnosed as an idiopathic mental retardation before this study, and they accounted for 8.6% of 152 males with mental retardation of unknown aetiology. Three index cases were isolated. All of the remaining nine cases from six families had one or more mentally retarded siblings; some cases also had other mentally retarded relatives. These six families were 22% of 27 families with a family history of idiopathic mental retardation in this population. In one family, a phenotypically normal male transmitted the fragile X syndrome to four grandsons and two granddaughters through five daughters. The frequencies of the fragile X syndrome in the retarded population reported here are similar to those in the retarded populations in the Northern Europe.

I-21. The Segregation Analysis of Four Families in Order to Know Genetic Effect on the Inducibility of Common Fragile Sites: **Yoshitsugu SUGIO and Yoshikazu KUROKI** (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

Frequencies of folate-sensitive common fragile sites (c-fra; 1p31, 1q44, 3p14, 3q26.2, 6q26, 16q23, Xp22.3) were examined in healthy 19 members from four families, ranging from 1 to 59 years old. They consisted of 12 males including a pair of identical twins and

7 females. Blood from the subjects was cultured for 96 hr in MEN without folic acid. Two hundred metaphases per one subject were scanned for a gap or a break, using conventional Giemsa staining. It's breakpoint was determined by the sequential Q-staining. In addition, we investigated whether breakages at 3p14 always occurred in the same chromosome 3, using Q-band heteromorphisms. Frequencies of c-fra showed individual differences between members of the same family and even between the identical twins. No data suggesting that the inducibility of c-fra was compatible with Mendelian codominant fashion were obtained. In nine subjects with two clearly fluorescent different chromosomes 3, roughly a half of all breakages at 3p14 occurred on the brightly fluorescent chromosome. This study suggests that the inducibility of c-fra is determined not only by structural factors on the site, but also by environmental factors *in vivo* and/or *in vitro*. Two previous studies on the relationship between inducibility of c-fra and genetic factors had reached different conclusions. The segregation analysis of families for levels of breakages at c-fra has not yet been reported.

I-22. An Osteogenesis Imperfecta with the High Frequency of Chromosomal Breaks and Gaps: **Hiromi SAKAMOTO, Tomoko HASHIMOTO, Yoshihiro YAMAMOTO, Jun-ichi FURUYAMA** (Dept. Genet., Hyogo Coll. Med., Nishinomiya), **Masanori TANIMURA, Kyoko ISHIMOTO, Hiroyoshi WADA** (Dept. Pediatr., Hyogo Coll. Med., Nishinomiya), **Satoko YOKOTA, Yoshie SUGAWARA and Norimitsu OHTSUKA** (Dept. Clin. Lab., Hyogo Coll. Med., Nishinomiya)

The patient was a 3-month-old boy. He was born at 39 weeks of gestation to unrelated parents. He was the fifth child of five sibs. His birth weight was 2,422 g. When he was one-month-old, he was admitted to a hospital owing to low body temperature. During the therapy for low temperature, bone fractures were noticed and referred to the Department of Pediatrics of our college. X-ray showed fractures of right tibia and left femur. The pediatricians diagnosed as osteogenesis imperfecta. His old looking face made us examine his chromosomes. In routine chromosomal examination from his peripheral lymphocytes after 72 hr incubation, some chromosomes had gaps and others breaks. His karyotype was a normal male type. We examined the chromosomes after 48 hr incubation and after incubation with mitomycin C (MMC) (50 ng/ml). The results showed a higher frequency of chromosomal aberrations. After 48 hr incubation, 0.088 breaks/cell could be seen and after 92 hr incubation with MMC 0.080 breaks/cell (each normal control 0.02–0.06 breaks/cell). The frequency of sister chromatid exchanges (SCE) was 4.20/cell and this is within normal limits. His RBC decreased in number but reticulocytes increased. His WBC and platelets were normal. The high frequency of chromosomal aberrations

made us suspect that he might be suffered from Fanconi's anemia. We are going to follow up this patient.

I-23. Fragile Sites on Cynomolgus Monkey Chromosomes: **Momoki HIRAI** (Dept. Anthropol., Fac. Sci., Univ. Tokyo, Tokyo), **Keiji TERAO, Fumiaki CHO and Shigeo HONJO** (Tsukuba Primate Cent., NIH, Tsukuba)

Fragile sites of the monkey chromosomes were analyzed. Blood samples from a total of 150 cynomolgus monkeys (*Macaca fascicularis*), composed of 90 females and 60 males, were cultured under the conditions known to reveal the fragile sites of human chromosomes. One individual was found to show a possible BrdU-requiring heritable fragile site on the No. 11 chromosome (a middle-sized submetacentric chromosome). This may lead us to expect some correspondence between the No. 11 chromosome and the human No. 10 chromosome. However, after banding analysis, this was found not to be the case. At least 6 common fragile sites (or hot spots) were detected. In particular, the No. 16 chromosome (a small submetacentric chromosome) showed distinct gaps or breaks under the folic acid-deprived condition. No distamycin A inducing fragile site was found. The breeding of monkeys bearing these fragile sites may facilitate the gathering of information on the mechanisms of expression of the fragile sites and their biological significance in carcinogenesis.

I-24. Epstein-Bar Virus Transformant with Reciprocal Translocation t(6;14): **Masayuki OTSU, Yoshio YAOITA** (Dept. Med. Chem., Kyoto Univ., Kyoto), **Shigeru KATAMINE** (Dept. Bacteriol., Nagasaki Univ., Nagasaki), **Masatsune UNO, Mitsuo YAMAKI, Yasushi ONO** (Dept. Microbiol., Nihon Univ., Tokyo), **M.S. SASAKI** (Rad. Biol. Cent., Kyoto Univ., Kyoto) **and Tasuku HONJO** (Dept. Med. Chem., Kyoto Univ., Kyoto)

Epstein-Barr virus (EBV) is a supposed causative agent of Burkitt's lymphoma. EBV-transformants, however, do not show malignant features like Burkitt's cells and the former with reciprocal translocations involving immunoglobulin (Ig) genes have not been reported. To clarify their differences, it is required to establish EBV-transformants with such translocations. Here, we describe an EBV-transformant with a reciprocal translocation occurred in Ig heavy chain gene. This cell line FLEB 14·Δ3 was identified as a cell line with aberrant reorganization of Ig heavy chain gene during cultivation of FLEB 14, an immature B cell line without Ig gene rearrangement. Molecular analysis of FLEB 14·Δ3 revealed the rearrangement occurred in S_μ region by non-homologous, reciprocal recombination with deletion of 9 base-pair sequence. Chromosomal analysis demonstrated 46,XY,t(6;14)

(q15;q23), where 14q32 is the locus of Ig heavy chain gene. Thus, FLEB 14·Δ3 is an EBV-transformant with analogous reciprocal translocation to those found in Burkitt's cells, though the counterpart of the translocation is from chromosome 6, not from chromosome 8.

I-25. A Simple Technique for Chromosome Preparations from Single Hemopoietic Colonies: **Yoshiaki SONODA, Shohei YOKOTA, Tsukasa OKUDA, Taira MAEKAWA, Masafumi TANIWAKI, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO** (3rd Dept. Intern. Med., Kyoto Pref. Univ. Med., Kyoto)

We report a simple technique for chromosome preparations from single hemopoietic colonies cultured in methylcellulose. On Day 7–14, individual colonies were aspirated using micropipette or micromanipulator. Method A: Colonies were transferred into 0.2 ml of PBS in microtiter well. After centrifugation, Colcemid (0.1 μl/ml) was added to each well and incubated for 30 min at 37°C. Then 0.2 ml of 0.06 M KCl solution was added and incubated for additional 20 min. Cells were resuspended in 0.02 ml of hypotonic solution and gently dropped onto poly-L-lysine (PLL) coated slide. Slides were allowed to stand for 10 min in a wet-chamber. Method B: Colonies were transferred into a droplet of PBS (10 μg) with Colcemid (0.1 μl/ml) on PLL-slide. Slides were turned upside down and allowed to stand for 30 min at 37°C in a wet-chamber. Slides were turned upright and added 30 μg of 0.05 M KCl. Slides were turned upside down again and stood for 15 min in a wet-chamber. Then slides were turned upright again and stood for 10 min in a wet-chamber. Fixation method was the same for methods A and B. Two to three droplets of fixative diluted with 0.075 M KCl (30→70→100%) were gently added to a droplet on PLL-slide. Slides were immersed into fresh fixative for 10 min and flame-dried. Scanning was performed after simple Giemsa staining and banding analysis using AMD/DAPI staining was also done after decolorization of slides.

I-26. 大腸腺腫症患者および正常人の培養線維芽細胞における染色体異常： **高井節夫[1]・外村　晶[1]・岩間毅夫[2]**（東医歯大・[1]難研，[2]二外）． **Chromosomal Aberrations in Cultured Skin Fibroblasts from Patients with Adenomatosis Coli and Normal Persons:** Setsuo TAKAI[1], Akira TONOMURA[1] and Takeo IWAMA[2] ([1]Dept. Cytogenet., Med. Res. Inst., [2]2nd Dept. Surg., Tokyo Med. Dent. Univ., Tokyo)

常染色体優性遺伝疾患で高発癌性の家族性大腸ポリポージス（FPC, 30人），Peutz-Jeghers 症候群（PJ, 10人）の患者および正常者（29人）由来の培養皮膚線維芽細胞における染色体異常を分析した．対象者 1 人につき 50 細胞ずつ観察し，通常のギムザ染色で構造異常を有すると判断された場合には，脱色後キナクリン染色し，異常染色体の同定を行った．結果は，構造異常を持った細胞の出現頻

度の平均は FPC (平均 32.1 歳) で 2.7% (0~10%), PJ (20.0 歳) で 7.8% (0~50%), 胎児由来の細胞 (5 人) で 0%, 20~50 歳 (30.4 歳, 14 人) の正常者で 1.3% (0~4%), 60~70 歳 (65.0 歳, 10 人) の正常者で 2.4% (0~6%) であった. FPC や PJ では, 同世代の正常者より相互転座などの構造異常を有する細胞が多くみられた. また正常者でも, 年齢が高くなるにつれて構造異常が出現することが判明した. なお, 異常染色体の切断点は, FPC では 1q1, PJ では 1p3, 正常者では 2q1 がもっとも多かったが, 切断点に部位指向性があるかどうかについてはさらに研究する必要がある.

I-27. Consistent Chromosome Abnormalities in Adult T-Cell Leukemia: Naoki SADAMORI, Miyuki KUSANO, Kenji NISHINO, Hideo NAKAMURA, Shuichi IKEDA, Saburo MOMITA, Kenichiro KINOSHITA and Michito ICHIMARU
(Dept. Intern. Med., Atomic Disease Inst., Nagasaki Univ., Nagasaki)

Adult T-cell leukemia (ATL) is a newly defined clinical entity on the basis of the clinical picture, characteristic morphology of the abnormal lymphocytes, T-cell markers, geopathologic distribution with a much higher frequency in southwest Japan, and HTLV/ATLV viruses. On the other hand, achievements in the cytogenetic studies of ATL are scarce. In the present study, we performed cytogenetic analysis of eight typical ATL patients. Leukemic cells for cytogenetic study were obtained from the peripheral blood of ATL patients at the time of diagnosis. Mononuclear cells separated by centrifugation with Ficoll-Hypaque gradients were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum but without any mitogens. The cells were harvested after 1–20 hr. Colcemid was added 1 or 2 hr before harvesting. In each specimen, at least 15 metaphases were analyzed using G- and Q-banding techniques. Abnormal clones existed in all the patients. The modal chromosome numbers ranged from 44 to 48. The chromosome banding pattern revealed the presence of an abnormal chromosome #14 with a break at band 14q11 in six out of eight patients with ATL. Two patients had an inversion of chromosome #14, inv(14)(q11q32), and two patients had a translocation between chromosome #14 and #11 or #14. Recently Ueshima *et al.* indicated that most reported patients with T-cell malignancies except ATL had a rearrangement of the long arm of chromosome #14 with a break at hand 14q11-13. The data of ATL presented by us support the finding of a break in the upper segment of chromosome #14 in T-cell malignancies reviewed by Ueshima *et al.* We suggest a possibility that the proximal 14q rearrangement at band 14q11 in T-cells associated with HTLV/ATLV is one of the essential factors for transformation to ATL.

I-28. A Cytogenetic Study of Chronic Lymphocytic Leukemia: Tamiko SHINOHARA (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent., Tokyo)

Chronic lymphocytic leukemia (CLL) is rare in Japan, and cytogenetic investigations of the leukemic cells have remained insufficient. In this report, a cytogenetic study on 8 Japanese patients with CLL was presented. Lymphocytes from 8 patients with CLL were stimulated by phytohemagglutinin (PHA), pokeweed mitogen (PWM) or Epstein-Barr virus (EBV). Chromosome analyses with trypsin-Giemsa banding technique were performed on peripheral blood lymphocytes after 3 or 5 days of incubation and on bone narrow cells after 17 hr of culture. Five of 8 cases were clinically and immunologically B-cell type CLL and 2 cases T-cell type CLL, whose ages ranged from 40 to 76 years. Diagnosis was based on accepted clinical, hematological and immunological criteria. White blood cell counts ranged from $8\times10^3/mm^3$ to $21\times10^3/mm^3$, and frequencies of lymphocytes ranged from 80 to 90% in 8 patients. Cytogenetic analysis revealed that one patient, diagnosed as B-cell type CLL, had a clonal abnormality in the leukemic cells. The abnormal karyotype was 46,XY,+3,t(10;19)(q22;p13),13q+,t(11;14)(q13;q32),−15,17q−. The frequencies of this abnormal cell were 50%, 13% and 100% in cells stimulated by PHA, PWM and EBV, respectively. The finding of the same clonal abnormality after stimulation with three different mitogens suggests that the factor responsible for the development of CLL might affect the stem cell common to T- and B-cell lymphocytes. In this abnormal karyotype, the 13q+ chromosome seemed to show homogeneous staining region (HSR). In the present series, trisomy 12 known to be a specific abnormality in B-CLL was not observed. Another patient showed a karyotype of 46,XY,inv(16)(p11.2q24) as a clonal abnormality.

I-29. 新しい転座染色体 t(9q;13q) を伴った急性骨髄性白血病の一例：近藤郁子[1]・石川敏子[2]・中沢正樹[2]・阿部 帥[2]（筑波大・[1]基礎医・人類遺伝，[2]臨床医・血液内科）．
A Case Report of a Patient with Acute Non-lymphoblastic Leukemia (M2 type) and t(9q;13q): I. KONDO, T. ISHIKAWA, M. NAKAZAWA and T. ABE (Univ. Tsukuba, Ibaraki)

myelodysplastic syndrome (MDS) はしばしば急性骨髄性白血病 (ANLL) に移行する造血病態であるが，高頻度に染色体異常を伴うことが報告されており，MDS から移行した AML は，その他の AML と異なった病態を示すことが知られている．本発表では，MDS から移行したと強く疑われた ANLL (FAB 分類 M_2) の症例の骨髄の染色体分析の結果，報告のない新しい染色体転座 t(9;13)(q34;q11) を認めたので，その臨床像と染色体異常を検討し報告する．症例は47 歳，女性で，昭和 60 年 5 月汎血球減少を指摘され筑波大学病院に入院した．末梢血は高度の汎血球減少を示し，芽球が 8% 認められた．骨髄では芽球と前骨髄球が 50% を占め，形態学的に異常な好中球，赤芽球および巨核球を認め，ANLL (M_2) と診断された．骨髄の染色体分析の結果，30 個の分析細胞すべて

が 46,XX,del(6)(p23), t(6;13)(q34;11) の核型を示した．薬剤三者併用療法，輸血の治療には反応せず1カ月後の病態，染色体所見は入院時と同様であった．ANLL (M_2) や MDS に固有の染色体異常の解析が進められているが，本症例の転座染色体の報告はない．本症例ではこの転座がいずれの病態に出現したものか現在不明であるが，今後の症例の集積により前白血病状態から白血病へのガン化に関与する染色体異常を明らかにしていくうえで重要な症例として報告した．

I-30. Cytogenetic Studies in Myelodysplastic Syndrome (IV): Prognostic and Clinical Significance of Chromosome Abnormalities: Masafumi TANIWAKI, Shigeo HORIIKE, Johji INAZAWA, Tsukasa OKUDA, Hiromi YASHIGE, Shohei YOKOTA, Kazuhiro NISHIDA, Yoshiaki SONODA, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (Dept. Med., Kyoto Pref. Univ. Med., Kyoto)

Cytogenetic analyses were done on 41 patients with myelodysplastic syndrome (MDS): 7 with refractory anemia (RA), 3 with RA with ring sideroblasts (RARS), 10 with RA with excess of blasts (RAEB), 12 with RAEB 'in transformation' (RAEB-T), 6 with chronic myelomonocytic leukemia (CMML) according to French-American-British (FAB) criteria, and additional 3 patients with MDS which was unable to be classified by FAB criteria (UMDS). Chromosome banding techniques showed clonal karyotypic abnormalities in 26 of 41 patients (63.4%). Seven of them (27%) showed either 5q−, 7q−/−7, or +1q, all belonging to RAEB-T or UMDS. Three of these 7 patients had previously been exposed to mutagenic/carcinogenic agents. Fourteen of 41 patients (34.1%) evolved into acute leukemia that was defined by FAB criteria: 1 patient with M1, 8 with M2, 4 with M4, and 1 with M6. The patients with a normal karyotype or a mixture of normal and abnormal karyotypes had longer survivals of 21 months (50% survival), with 15% (4/25) undergoing leukemic transformation, than those with only abnormal metaphases (6 months, with 62.5% (10/16) acute transformation). Thus, residue of cytogenetically-normal clone indicates a favorable prognosis.

I-31. Cytogenetic Studies of Renal Cell Carcinoma: Mitsuaki A. YOSHIDA (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo) **and Avery A. SANDBERG** (Dept. Genet. Endoclin., Roswell Park Memorial Inst., Buffalo, USA)

Cytogenetic analysis was successfully done in fourteen tumor materials from 12 patients with non-familial renal cell carcinoma. A combined method of enzymatic technique and short term culture was applied to these tumor tissues. Modal chromosome number was shown in the range of 38–46 in 11 samples and 72 in one case, and it was not decided in the remaining two samples. Q- and G-banding analysis revealed 45 clonal aberrations in 11 tumor samples from 10 patients. The rearrangements of chromosome #3 were involved in 12 (26.6%) of these aberrant clones and were found commonly in 8 of 12 patients ex-

amined, *i.e.*, one case of t(3;8)(p14-21;q24.1) and del(3)(p14-21), three cases of del(3p) with different breakpoints (p11-14, p12, p14-21), one case of isochromosome for a short arm of #3 and del(3)(p14-21) and two cases of t(3;5) with different breakpoints (p11,q13.2). The breakpoints of these clonal rearrangements of #3 were clustered in the region of p11-21 in seven cases except for one case with the translocation between #3q and #5p. One case in which two tumor tissues were obtained from both primary and metastastic sites showed isochromosomes for both arms of #1 in metastasized tissue. Two cases did not show any clonal abnormalities. The remaining one case had one clonal change, deletion #6q. On the other hand, chromosomes #1, 2, 6, 11, and 17 were frequently involved in the remaining 33 out of 45 abnormal clones. Thus, the chromosome rearrangements of #3 were commonly identified in 8 cases out of 12 patients with non-familial renal cell carcinoma. These results suggest that renal cell carcinoma is cytogenetically classified into three categories: the tumor 1) with chromosome changes of #3, 2) with other clonal abnormalities and 3) without clonal changes, and that the rearrangements of chromosome #3 may be possibly associated with the genesis and/or progression of renal cell carcinoma as in the cases with the familial form.

I-32. High Resolution G-Banded Analysis in Aniridia: **Yoshihiro HOTTA,[1] Haruko SHIRATO,[1] Keiko FUJIKI,[1] Nobuo ISHIDA,[1] Kazuo KATO,[1] Akira NAKAJIMA[1] and Hideo TAKAMATU[2]** ([1]Dept. Ophthalmol., [2]Dept. Pediatr. Surg., Juntendo Univ., Tokyo)

Chromosomes of 8 patients with aniridia were studied by high resolution G-banded analysis (750 bands). We found the deletion of the short arm of chromosome 11 in two cases, del(11)(p13p14) and del(11)(p11.2p14.2), respectively. One of these cases was a three months old boy, and Wilms' tumor was suspected. Elaborate investigations were performed by the pediatric surgeons. Wilms' tumor was found in his left kidney and was successfully removed in early stage. The other case, 3 year old girl, did not have Wilms' tumor. These results suggests that the high resolution G-banded analysis should be performed in all cases with aniridia in infant as a screening test to find out Wilms' tumor as early as possible.

I-33. Gene Dosage Effects of Esterase D (ESD) in a Case with 13q Interstitial Deletion, del(13)(q12.3q14.11): **Ryozo KASAI, Yoko NAKASHIMA, Masae MURAKAMI** (Asahigawa Jidoin Child. Hosp., Okayama), **Kouji NARAHARA, Yukio TAKAHASHI, Yoshiharu WAKITA, Kiyoshi KIKKAWA, Shunsuke KIMURA and Hiroshi KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama)

The genes coding for esterase D (ESD) and retinoblastoma (RB), which have been mapped to 13q14.1 (HGM 7, 1984), are known to be linked closely to each other. The accurate localizations of these genes on 13q14.1 have been undetermined yet. Sparkes *et al.* (1984) have recently suggested that in this region ESD is more proximally located than RB. We present here a case with an interstitial deletion of 13q in which a gene dosage study of ESD was conducted. The case, a 6 year and 6 month-old girl, had clinical features similar to those of previously reported cases with a proximal 13q monosomy. Repeated ophthalmologic examinations revealed no retinoblastoma. The karyotype identified by the high-resolution banding method was 46,XX,del(13)(q12.3q14.11) *de novo*. Analyses of QFQ-heteromorphisms disclosed that the deleted chromosome 13 of the case had originated from a paternal chromosome 13. On the other hand, the father and the paternal grandfather were found to have an extra chromosome material on the distal 8p portion, which were too minute to identify the exact nature. Studies of the ESD activities and phenotypes revealed the case: 3.93 U/gHb (type 1), the mother: 2.88 U/gHb (type 2-1), and the father: 3.81 U/gHb (type 1), indicating normal gene dosage for ESD in the case. These results suggest that ESD can be excluded from 13q12.3→q14.11, and that the gene dosage study of ESD is useful to predict the RB in patients with proximal 13q deletions involving 13q14.1.

I-34. Familial Retinoblastoma with 13q−[del(13)(q14.3q22)] and Normal Esterase D Activity: **Yoshimitsu FUKUSHIMA,[1] Yoshikazu KUROKI[1] and Taizoh ITO[2]**
([1]Div. Med. Genet., [2]Div. Ophthalmol., Kanagawa Child. Med. Cent., Yokohama)

The proband is a 1 9/12 year-old boy who was found to have unilateral retinoblastoma at 1 1/12 years of age. His developmental milestones were delayed (DQ: 76). His facies was characterized by a high forehead, a low and broad nasal root, a short and bulbous nose, a long philtrum, an open mouth with a upper lip and prominent earlobes. His father was 34 years old and was affected with myopic amblyopia but he was mentally normal and had no malformations. The mother was 33 years old and she had suffered from retinoblastoma in the right eye at 6 months of age. She grew up with normal mentality. Her facies resembled that of the proband: a high forehead, the low and broad nasal root, a short and bulbous nose, a long philtrum, an open mouth with a thin upper lip and prominent earlobes. She was healthy and had no other malformations nor diseases except for visual disturbance. High-resolution chromosome banding analysis with the 550 to 850-band stage revealed that both the proband and his mother had the same interstitial deletion of the long arm of chromosome 13 resulting from a loss of a subband q14.3 through q22.3 [46,XY or XX,del(13)(q14.3q22.3)]. Esterase D activity on fibroblasts from the proband and his mother showed a type 1-1 with 34.46 units and a type 1-1 with 31.13 units, respectively, which are within the normal range. This family is the first instance of dominantly

inherited retinoblastoma that is affected with 13q−, and indicates that retinoblastoma gene is located at 13q14.3.

I-35. Cytogenetic Studies on the Development of Retinoblastoma: Masao S. SASAKI, Yosuke EJIMA (Radiat. Biol. Cent., Kyoto Univ.), **Akihiro KANEKO** (Natl. Cancer Cent. Hosp., Tokyo) **and Hiroshi TANOOKA** (Natl. Cancer Cent. Res. Inst., Tokyo)

Chromosome constitutions of somatic cells were studied in 176 patients with retinoblastoma (RB). Constitutional chromosome abnormalities were found in 12 patients, including 8 cases with del (13), one with t(13,X) and 3 cases with autosomal translocation occurring at 13q14.11. These abnormalities were consistent in that a disturbance of normal expression of gene(s) at 13q14.11 links to the development of RB. These chromosome mutations constituted 14.1% of newly arising germinal mutations. The origin of these chromosome aberrations was studied by fluorescence markers and/or electrophoretic polymorphism of esterase D. Out of 8 informative cases, 7 were paternal and one was maternal in origin. In the analysis of distribution of age at first diagnosis, a fraction of patients not diagnosed (S) by age M in month showed the best fit to a simple exponential function of $S=\exp(-aM)$ both in bilateral and sporadic unilateral cases, and coefficient, a, was 0.046 ± 0.085 for sporadic unilateral cases and 0.130 ± 0.222 for bilateral cases. Lack of difference in the age exponent between two types of RB, indicate that the RB mutations occur before birth, probably before the differentiation of retina, in both types of RB. Chromosomes were also studied in 30 cases of RB primary tumors. The presence of +1q or +i(6p) was common and appeared in 28 tumors. The +1q occurred as an extra chromosome material translocated onto other chromosomes. When cells were classified according to the types of appearance of +1q, the cell populations appeared monoclonal in sporadic unilateral cases while they were polyclonal in bilateral cases. This indicates that the generation of +1q aberration plays a significant role in engendering tumorigenicity when cells have on RB mutation already.

I-36. Use of Retinoblastoma and Wilms' Tumor as Sentinel Phenotypes for Population Surveillance: Ei MATSUNAGA (Natl. Inst. Genet., Mishima) **and Kensei MINODA** (Tokyo Metropol. Yoiku-in Hosp., Tokyo)

A vast majority of retinoblastoma (RB) and Wilms' tumor (WT) occur sporadically, and they are generally arising from a germinal or somatic mutation. In countries where systematic registration of childhood malignancies has been established, data on the incidence of the two tumors may be used for pupulation surveillance of environmental mutagens.

In Japan, the ongoing nation-wide registration of all childhood malignancies is supported by a private foundation and maintained by voluntary cooperation of physicians. The current data are, however, not satisfactory because of underreporting (Hanawa, Y., 1975), with exception of those for RB reported in the initial stage of registration (Minoda, K., 1976). On the other hand, fairly reliable data at the prefectural level are available in Kanagawa, where the ascertainment is made by reviewing the application form submitted by the patients to the local government for defraying the cost of medical treatment (Nishihira, K., 1983); since 1971 childhood malignancies have been among a number of "designated diseases" for which, upon application, such assistance is rendered by central and local government. From these data the live-birth frequencies of RB and WT are estimated to be about 1:15,000 and 1:20,000, respectively, in Japan.

With approximately 1.5 million births a year, the annual number of new cases of the two tumors combined would be about 175. If this is taken as a standard, then the border-line above which a rise in the incidence is statistically significant at the 1% level would be 210. If a significant rise in the incidence of sporadic cases could be detected, a case-control study has to be undertaken. In bilateral cases either paternal or maternal exposure prior to the conception of the index child should be suspected, whereas in unilateral cases the child's exposure before or after birth should be suspected. The Ministry of Health and Welfare should encourage and promote the registration of childhood malignancies on the population basis.

1-37. Supression of the *c-myc* Expression in a Mouse Plasmacytoma Line by Fusing Normal Fibroblasts: **Tsuneyuki OIKAWA, Nobuo KONDOH, Naoya YUHKI, Seiji YAMAGIWA, Noboru KUZUMAKI** (Lab. Genet., Cancer Inst., Hokkaido Univ. Sch. Med., Sapporo), **Yuhko YUHKI** (Dept. Pediatr. Stomatol., Hokkaido Univ. Sch. Dent., Sapporo) **and Kenji ABE** (Chrom. Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

The S194, a BALB/c mouse plasmacytoma line, shows an elevated level of the *c-myc* mRNA, having a specific translocation between chromosomes 12 and 15. To elucidate the *c-myc* regulation in mouse plasmacytomas, we fused S194 cells with two different cell leanages of normal spleen cells or fibroblasts. Hybrid clones were selected HAT-ouabain medium in 96 well plastic plates. The clones retaining almost all chromosomes from the both parents were selected for Southern or Northern analysis using an *Eco*RI/*Cla*I fragment of the human *c-myc* exon III as a probe. The hybrid clones between S194 cells and normal spleen cells showed the same level in the *c-myc* expression as the parental S194 cells. On the other hand, the hybrid clones between S194 cells and normal fibroblasts showed reduced level in the *c-myc* expression compared with the parental S194 cells, although

they were proven to retain the rearranged *c-myc* by Southern analysis. These results indicate that a trans-acting suppressive factor(s) for the *c-myc* expression in mouse plasmacytomas may exist in normal fibroblasts.

I-38. Expression of *c-Ha-ras* and *c-myc* Genes in Familial Polyposis Coli: Kenji SUGIO, Takehiko SASAZUKI (Dept. Genet., Kyushu Univ., Fukuoka), **Shun-ichi KURATA** (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo) **and Takeo IWAMA** (Dept. Surg., Tokyo Med. Dent. Univ., Tokyo)

Familial polyposis coli (FPC), which is an autosomal dominant trait and which is at high risk colon cancer, is a good model to understand the process of carcinogenesis. We examined the expression of *c-Ha-ras* and *c-myc* genes using the dot blot and Northen blot technique in adenomatous polyps, cancer and normal colorectal mucosa in three patients with FPC. A *c-Ha-ras* gene related transcript of 1.5 kb was detected and its expression was 10 fold elevated in premalignant and malignant tissues compared with normal colorectal mucosa in three patients with FPC. A *c-myc* gene related transcript of 2.5 kb was detected and its expression was 2–4 fold elevated in premalignant tissues and 5 fold elevated in malignant tissues compared with normal colorectal mucosa in two patients with FPC. The elevated expression of these genes in premalignant state is possibly one of the factors involved in carcinogenesis. Southern blot analysis did not reveal amplification or rearrangement of *c-Ha-ras* and *c-myc* genes. By using NIH/3T3 as a recipient cell, no transforming genes were detected in DNA extracted from these tissues in two patients with FPC.

I-39. Establishment of Cell Lines from Ataxia Telangiectasia and Xeroderma Pigmentosum Diploid Fibroblasts by Oncogenes: Tomoko HASHIMOTO (Dept. Genet., Hyogo Coll. Med., Nishinomiya), **Yoshiro NAKANO, Takeo KAKUNAGA** (Dept. Oncogene Res. Inst. Microb. Dis., Osaka Univ., Suita), **Koji OWADA** (Dept. Tumor Virol., Res. Inst. Microb. Dis., Osaka Univ., Suita), **Yoshihiro YAMAMOTO and Jun-ichi FURUYAMA** (Dept. Genet., Hyogo Coll. Med., Nishinomiya)

Ataxia telangiectasia (AT) and xeroderma pigmentosum (XP) are syndromes characterized by DNA repair defficiency, patients being prone to develop various cancers. In ordinary cell culture conditions, human fibroblasts do not acquire immortality spontaneously. We were able to establish fibroblast cell lines from AT and XP patients by transfection of viral oncogenes, pSV40 (SV40 whole genome inserted into pBR322) or pSV40 plus pMoMSV (Moloney mouse sarcoma virus whole genome inserted into pBR322). Three lines of AT

and one of XP were immortalized: AT-T28 and AT-T55 were established by transfection of pSV40 and pMoMSV, AT-T53 and XP-T11 by transfection of pSV40. AT-T28 was maintained for 20 months and population doublings were 280 after transfection. These four lines showed transformed phenotype (morphological change, anchorage independency, increased cell density, and serum independency) keeping original characteristics (identical heteromorphisms of the chromosomes revealed using Q-banding and hypersensitivity to γ-ray or ultraviolet), but no tumorigenicity in nude mice. Cellular DNA was analyzed by Southern blotting. All four line DNAs digested with restriction enzymes showed the band hybridized with pSV40 but the pattern was different in all of them. When mos DNA was used as a probe, these four line and non-transformed fibroblast DNAs showed the same pattern of hybridization. These results indicate that pSV40 transfection was essential for establishment of the cell lines in our system.

I-40. Molecular Evidence for the Lack of EGF Receptor Gene Expression in Human Small Cell Lung Carcinoma Cells: **Shinobu GAMOU,[1] John HUNTS,[2] Harumi HARIGAI,[1] Setsuo HIROHASHI,[3] Yukio SHIMOSATO,[3] and Nobuyoshi SIMIZU[1,2]** ([1]Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo, [2]Dept. Mol. Cell. Biol., Univ. Arizona, Tucson, [3]Div. Pathol., Natl. Cancer Cent. Res. Inst., Tokyo)

It has been shown that none of the small cell lung carcinoma (SCLC) cell lines possess EGF binding activity on their surface. We have examined several SCLC cell lines H69, H128, Nat231, Lu134 for the possibility that they may have EGF receptors but that the receptors are masked by an EGF-like protein factor(s) which may be produced by an autocrine machanism. No evidence was found for the production of such factors. We then used an EGF receptor cDNA to determine the state of the EGF receptor gene by Southern blot analysis. The receptor gene appears to be present in these cells in an intact, unrearranged form. These cells, however, were found to be deficient in detectable levels of EGF receptor mRNA, revealing the reason for no EGF receptors on the cell surface. Furthermore, karyotype analysis revealed that Lu134 and H69 contained a morphologically normal chromosome 7, which carries the EGF receptor gene. These results indicate that SCLC cells have some active regulatory mechanism which prevents EGF receptor expression. These cheracteristics of SCLC may help to better define their tissue of origin. In this study, we were not able to confirm the previous finding that the SCLC cells have in common the partial deletion of the short arm of chromosome 3.

I-41. 脂漏性角化症（老人性疣ぜい）の細胞遺伝学的検討：平本道昭[1]・林　研[2]・井階幸一[3]（京大・医・[1]形成外，[2]産婦人，[3]皮膚），**山本純子**（倉敷中央病院・細胞遺伝検）．**Cytogenetic Studies on Seborrheic Keratosis (Verucae Senilis):** M. HIRAMOTO, K. HAYASHI, K. IKAI (Kyoto Univ. Hosp., Kyoto) and J. YAMAMOTO (Kurashiki Cent. Hosp., Kurashiki)

脂漏性角化症（以下，SK）は黒褐色の扁平隆起した小腫瘤で，ケラチノサイトの局所的な成熟遅延による良性の表皮性腫瘍であり，50 歳以上の高齢者では 80% 以上にみられるきわめて頻度の高い疾患である．悪性化はしないという見解が現在支配的であるが，SK が悪性化した症例は稀ではあるが報告されている．悪性化に際しては基底細胞癌に変化する場合が多く，有棘細胞癌はさらに少ない．11 症例 14 個所の腫瘤および全症例の末梢血の染色体分析を行った．正常 7 個所，異常 7 個所で，1 症例より 2 個所検査したのではおのおのが正常および異常 1 個所ずつであった．末梢血では，すべて正常であった．病理組織学検査では，14 個所とも悪性像はみられなかった．異常症例のうち 2 症例において，核型：46,XY, t(9;11)(q12;p13) および 46,XY, t(7;11)(p14;p15) がみられ，No. 11 短腕部に切断点をもつ transposition の共通な変化が認められた．これは，oncogene の一つである *HRAS* 1 との関係が指摘されている位置の変化と考えられる．以上，いまだわずかな例数でしかないが，今後さらに症例を増していき，共通した染色体異常の存否を求めていきたい．

I-42. Immunological Study of Monozygotic Twins by Flow Cytometry: Yukio SATOW,[1] Naomasa OKAMOTO,[1] Noritaka IMAMURA,[2] Shizuyo KUZUMI,[2] Atsushi KURAMOTO,[2] Shoji WATANABE,[3] Masaki MUNAKA[3] and Minoru KURIHARA[3] ([1]Dept. Geneticopathol., [2]Dept. Intern. Med., [3]Dept. Biometr. Soc. Med., Res. Inst. Nucl. Med. Biol., Hiroshima Univ., Hiroshima)

Purpose: On 9 families comprising 26 cases including 9 pairs of M.Z. twins (ages 20–64, 6 male and 3 female pairs), an analysis was made on T, B lymphocyte subsets employing a cell sorter, using monoclonal antibodies (OKT 3, 4, 8, 11 and B-1, *etc*)., to study the hereditary background of human immune competence. *Results and Discussion:* (1) Among 9 pairs of twins, 5 pairs presented abnormally high T4/T8 ratios (2.5–4.7) due to increase of T4-positive helper/inducer cells and decrease of T8-positive suppressor/cytotoxic cells. Three pairs had normal values for both of the twins, and one pair showed an abnormally high value for one of the twins. (2) All of the 4 children born of two parent pairs of twins presenting high T4/T8 ratios showed normal values. (A) Among 3 pairs of twins of the next generation of 3 parents who showed high T4/T8 ratios, 2 pairs of twins presented abnormally high values and one pair of twins presented normal values. The above findings suggest that the offspring are not necessarily abnormal even though abnormally high values are presented by the parent twins and that there is a possibility in parents when high values are presented by both of the twins. Cases presenting high T4/T8 ratios were considered to be a latent immunologically unbalanced condition because no autoimmune

diseases were found, but the T4/T8 ratios of twins showed high rates of concordance for normal or abnormal values.

I-43. Nature/Nurture Problem in Parkinson's Disease: Report of a Case-Control Study: Kiyotaro KONDO (Dept. Public Health, Hokkaido Univ., Sapporo)

A multifactorial causation was proposed by a family study (Kondo *et al.*, 1973), but recently NIH data of the twins disclosed a rather low concordance rate, 4/48 in M2 and 1/19 in DZ, heritability being only 0.22. Our collaborative case-control study involving 166 cases matched with three each controls revealed "rigid" personality profile, non-smoking and habitual constipation as the risk factors to the disease. A multivariate logistic model showed that combinations of two or more these factors increased the relative risks up to 30 times over those having no such factors. These results supported a multifactorial hypothesis and suggested that more specific identification of manageable environmental risk factors may give clues to prevent the disease.

I-44. 遺伝と環境のからみあい：寺脇 保（鹿児島大・医・小児）. **Heredity and Circumstance:** Tamotsu TERAWAKI (Dept. Pediatr. Fac. Med., Kagoshima Univ., Kagoshima)

[研究方法] 遺伝と環境の疫学的研究を行った. [研究結果] 1) 世界一長寿者 (120 歳) S.I. 氏の家系は，両親もきょうだいも 70 歳以上であった. その甥姪その子らも死亡例なし. S.I. 氏は染色体正常，身長 143 cm，体重 43 kg，視力 (左角膜混濁，右正常)，聴力良好，血中の蛋白，脂肪，糖正常，白血球 5,500/mm^3，赤血球 324 万/mm^3，Hb 11.1 g/dl，IgG/500 IgA 391，IgM 114，IgD2，以下 C_2 72，C_4 26 (mg/dl). その他 20 数種の検査正常. 地区の気温は年間平均 22°C，冬の最低気温 8°C，地区住民 11,000 人中 90 歳以上 60 人. 2) 政治家の家系 H 家. H 家は 4 代にわたり東大法科出身で初代衆議院議長，2 代総理大臣，3 代外務大臣，4 代は 30 台で政務次官とすぐれている. 初代の妻は松本藩重役の娘で一流女子大学出身の女傑，2 代の妻は母の姪の子で父は高級官僚で一流女子大学出身，3 代の妻は一流実業家の娘で一流女子大学出身. 3 代の妻はいずれも賢夫人である. 今後，このように長寿者やすぐれた社会人の遺伝と環境のからみあいの研究をつづけたい.

I-45. 全身性エリテマトーデス患者および家族における赤血球 C3b レセプター活性：川口達大・横田英介・内藤 靖・成富由司・草場公宏・今村 孝（九大・医・一内）. **Erythrocyte C3b Receptor Activity in Patients with Systemic Lupus Erythematosus and Their Families:** T. KAWAGUCHI, E. YOKOTA, Y. NAITO, Y. NARITOMI, T. KUSABA and T. IMAMURA (1st Dept. Med., Fac. Med., Kyushu Univ., Fukuoka)

ヒト赤血球膜には，C3b に対するレセプター (CR_1) が存在するが，全身性エリテマトーデス (SLE)

患者において，遺伝的に CR_1 活性低下を示す例が増加している．今回われわれは，福岡集団における SLE 患者とその家族の CR_1 活性を測定し，CR_1 活性低下と SLE 発症の遺伝要因との関連について検討した．赤血球 CR_1 活性は，免疫粘着反応を用いて測定した．CR_1 活性低下は，SLE 患者で 101 例中 80 例 (79%)，正常者では 169 例中 22 例 (13%) に認められ，SLE 患者では正常者に比べて CR_1 活性低下の頻度が高かった．SLE 患者 14 家系について，患者家族の CR_1 活性を測定した．CR_1 活性低下が同一家系内に複数にみられるなど，CR_1 活性低下の遺伝性が示唆されたが，家系数が少なく，明らかな遺伝性を証明することはできなかった．今後は，正常者ならびに SLE 患者の家系の症例数をふやし，赤血球 CR_1 活性低下の遺伝性について検討していく予定である．

I-46. 膠原病の4家系と免疫遺伝学的マーカー：山口雅也・森戸文隆・兼岡秀俊・永吉敏郎 (佐賀医大・内科). **Four Pedigrees of Collagen Diseases and Immunogenetic Markers in the Family Members:** M. YAMAGUCHI, F. MORITO, H. KANEOKA and T. NAGAYOSHI (Dept. Intern. Med., Saga Med. Sch., Saga)

自己免疫疾患がポリジーン形質であることは，双生児・家系分析・集団調査および動物の交配実験より明らかである．そして各種の異なった自己免疫疾患が，同一の個体または近親の間で重複して発生するとこがしられているが，SLE と DM の同胞例の報告は稀である．今回われわれは，1) SLE と DM, 2) SLE と SLE/DM, 2) SLE と RA, 4) PSS と RA が発症した 4 家系を報告した．このような多発家系や重複症例の存在は，これらの自己免疫疾患が，ポリジーン系のなかの一部の遺伝子を共有していることを示しているが，DM や PSS の発症には，さらにその特異性を決定する因子が必要であると思われる．SLE は，これらの疾患のなかでもとくに重複例や家族発生率が高く，代表的な自己免疫疾患である．そこで現時点において，SLE の発症に関与していると思われる遺伝子または遺伝マーカーとして，C3b レセプター，HLA および Gm 関連遺伝子，さらに二次元電気泳動によって検出される Lp1, Lp17, S_1 の 3 種の遺伝子産物 (第 27, 28 回本大会にて発表) の 6 種をとりあげ検索した．第 1 家系では，これらのマーカーは発症の状況ないし抗体保有状況とよく一致した．第 2 家系についても，発病者は健常者に比較して明らかに高い score を示した．第 3 家系については，死亡者や不明者が多く data が不十分であるが，これら 6 種のマーカーが SLE の解析に有用であることがわかった．

I-47. The Frequencies of the Chromosomal Aberration and the Sister Chromatid Exchange in Lymphocytes from Twins: Kunihiko MIURA,[1] Kanehisa MORIMOTO,[1] Akira KOIZUMI,[1] Ichiro YAMADA[2] and Akio ASAKA[2] ([1]Dept. Public Health, [2]Dept. Mental Health, Univ. Tokyo, Tokyo)

Whole blood was drawn from monozygotic (pairs; male, 5 and female, 8) or dizygotic (pairs; male, 3) twins (aged 11–12 years old) and their mothers. Macro cultures have been done for each sample for 52 hr for the chromosomal aberration (CA) analysis, and for 72 hr with bromodeoxyuridine (40 μM) for the sister chromatid exchange (SCE) analysis. The cells for CA analysis were also exposed to γ-rays (2 Gy; dose rate, 0.5 Gy/hr) only

or γ-rays and cytosine arabinoside (ara-C; 5×10^{-5} M) simultaneously at G_0 phase. The cells for SCE analysis were also treated with mitomycin-C (MMC; 3×10^{-8} M) or 4-nitroquinoline 1-oxide (4NQO; 40 ng/ml) for the entire culture. The frequencies of the dicentric and ring (D+R) chromosomes and the SCEs were investigated on the air-dried samples. The D+R frequencies in cells exposed to γ-rays only or γ-rays plus ara-C showed no significant difference regarding to the zygosity. The SCE frequencies in non-treated, or MMC- or 4NQO-treated cultures also showed no difference regarding to the zygosity. Snedecor and Cochran's inter-class correlation coefficients between twin childrens showed no consistent tendency. SCE frequencies between twin children and their mothers showed no difference in zygosity.

I-48. Sister Chromatid Exchanges (SCEs) and Cell Cycle Kinetics in Human Lymphocytes Cultures Exposed to Theophylline: **Kumiko IIJIMA, Hiroaki NOBUHARA, Munehiro HIRAYAMA** (Dept. Maternal Child Health, Univ. Tokyo, Tokyo) **and Makoto HIGURASHI** (Dept. Public Health, Yamanashi Med. Sch., Yamanashi)

Cytogenetic assay systems based on the detection of sister chromatid exchanges (SCEs) are widely advocated as a sensitive screening method for assessing genotoxic potential. While many agents have been examined for their own ability to induce SCEs, information of their combined effects and dose-response have been few. We have examined the ability of theophylline to induce SCEs and it's combined effects with mitomycin C (MMC). Heparinized human peripheral blood was cultured in the medium containing 40 μM BUdR. Two experimental sets of cultures were incubated continuously in the presence of various concentrations (0–100 μg/ml) of theophylline. One set of cultures was exposed initially to MMC (3×10^{-6} M) for 1 hr immediately before adding PHA. Harlequine chromosomes were obtained by the FPG method. Treatment with theophylline induced SCEs in a dose-dependent manner and theophylline treatment combined with MMC also induced SCEs in the same manner: calculated linear regression lines were $Y=8.6+0.03X$ and $Y=32.9+0.1X$ (Y, SCEs per cell; X, dose of chemicals). Proliferative inhibition occurred during exposure to therophylline as shown by a decline in the proportion of the third or subsequent generation metaphase cells concomitant with an increase in the proportion of the first generation metaphase cells.

I-49. Mutagenicity of Immunosuppressants: II. Comparison of Sister Chromatid Exchange Inducibility between Ciclosporin and Other Immunosuppressants: **Kenji YUZAWA,[1] Katashi FUKAO,[1] Yoji IWASAKI,[1] Ikuko KONDO[2] and Hideo HAMAGUCHI[2]** ([1]Dept. Surg., [2]Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Multiple factors may be involved in the etiology of the high risk of malignant neoplasms in organ transplant recipients and the oncogenic effects of immunosuppressive agents as mutagens might be one of the factors. We have reported that Ciclosporin (CYA) induces sister chromatid exchange (SCE), though it was reported that CYA had no mutagenic effects in various experimental systems. In this study, we compared SCE inducibility between CYA and other immunosuppressive agents: Breidinin (BRD) and Methotrexate (MTX). Heparinized peripheral blood lymphocytes from a healthy adult were cultured in the presence of immunosuppressants for 2 hr. The PHA-P and BrdU were added to the culture medium and the cells were further cultured in the dark for 5 days. Lymphocytes were harvested by the usual procedure. After FPG staining, frequencies of SCE were counted. SCE frequencies increased significantly in the lymphocytes treated with any of immunosuppressants used. At the same concentration of 0.25 μg/ml, SCE frequencies increased most in the lymphocytes treated with MTX, and least with CYA. Considering the optimal blood levels in clinical use, SCE inducibility of CYA is less than that of BRD and MTX. CYA may have less mutagenicity than BRD and MTX.

I-50. Sister Chromatid Exchange (SCE) Induction by Halogenated Pyrimidines in Cultured Bloom's Syndrome (BS) Fibroblasts: **Syuiti ABE** (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

The inducibility of SCEs by halogenated pyrimidines including 5-bromo-2′-deoxyuridine (BrdUrd), 5-iodo-2′-deoxyuridine (IdUrd), 5-bromo-2′-deoxycytidine (BrdCyd), and 5-iodo-2′-deoxycytidine (IdCyd) was studied in cultured BS fibroblasts (BS2KA cells), to examine if basal SCE frequency in BS cells is associated with the pyrimidine base analogue employed for sister chromatid differentiation (SCD). In BS2KA cells, lowest concentration necessary for SCD was 0.05 μg/ml for both BrdUrd and BrdCyd, 0.5 μg/ml for IdUrd, and 1 μg/ml for IdCyd, respectively. When compared the SCE yields at dose range from these concentrations to the highest dose tested (20 μg/ml) of the 4 pyrimidines, the most potent SCE inducer was BrdUrd (41–69 SCEs/cell), followed in order by IdUrd (34–50 SCEs/cell), BrdCyd (31–49 SCEs/cell), and IdCyd (32–42 SCEs/cell). It should be mentioned, however, that the SCE frequency remained plateau at a dose range below 0.5 μg/ml of BrdUrd (41–42 SCEs/cell) and BrdCyd (31–32 SCEs/cell) and over 5 μg/ml of IdCyd (41–42 SCEs/cell). The present results show that the basal SCE frequency in BS cells is dependent on the pyrimidine analogue used for SCD, and that BS cells may intrinsically have a high level of basal SCEs.

I-51. Sensitivity to Cross Linking Agents of Fanconi Anemia Lymphocytes: **Takahiko SUKENAGA,[1] Tomoko HASHIMOTO,[2] Noriko MATSUMOTO,[2] Kiyoshi NAKAMURA,[2] Kazue NAKAMURA,[2] Yoshihiro YAMAMOTO,[2] Hiromi SAKAMOTO[2] and Jun-ichi FURUYAMA[2]** ([1]Dept. Radiol., [2]Dept. Genet., Hyogo Coll. Med., Nishinomiya)

FA2NI, 10 year-old male, was the first child of the unrelated parents. His younger sister died of aplastic anemia. The onset of aplastic anemia of FA2NI was 6 years of age. He came to Osaka National Hospital because of hemorrhagic diathesis at the age of 10 and his blood figure showed aplastic anemia. He had hypoplastic right thumb and the right radial bone was deleted. The clinical diagnosis was Fanconi anemia. Sensitivity to cross linking agents (mitomycin C, MMC, and diepoxybutane, DEB) were investigated using PHA-stimulated peripheral blood lymphocytes (PBLs) from the patient and Epstein-Barr virus-transformed lymphoblastoid cell lines (B-LCLs). PBL, cultured as whole blood, showed high frequency of chromosomal aberrations, but these chromosomal aberrations were not induced by MMC (96 hr culture, base line; 0.34, MMC 50 ng/ml; 0.38 breaks/cell) or DEB. However, B-LCLs proved to be highly sensitive to these agents when tested by chromosomal aberration induction and survival cell count. Then, MMC induction of the chromosomal aberrations of FA2NI-PBLs separated by Ficoll-Conray solution were studied. Chromosomal aberrations of FA2NI-PBLs were highly induced by MMC (base line; 0.75, MMC 50 ng/ml; 2.40 breaks/cell). These results indicate that the sensitivity to cross linking agents depends on culture conditions.

I-52. A群色素性乾皮症細胞における欠損因子について：**山泉　克・菅野辰生・岡田善雄・内田　驍**（阪大・細胞工学センター）**. Deficient Factor in the Cells from Patients with Xeroderma Pigmentosum Group A:** K. YAMAIZUMI, T. SUGANO, Y. OKADA and T. UCHIDA (1st Mol. Cell. Biol., Osaka Univ., Suita)

色素性乾皮症（XP）は常染色体性劣性の遺伝病で，紫外線による DNA 傷害の修復機構の欠損によることが示されている．現在 A 群から I 群までの 9 群の遺伝的相補性群が確定されているが，分子レベルでの研究は進んでいない．われわれは，正常ヒト細胞より得た抽出液を XP 細胞に注入すると，これらの XP 細胞に除去修復能が回復することを見いだし，これを指標として個々の欠損因子の精製を試みている．今回は，A 群の欠損因子（A 因子）について次のような性質を明らかにすることができたので報告する．1) A 因子はプロテアーゼ処理により失活するが，RNase 処理には抵抗性である．2) A 因子を注入された A 群 XP 細胞は，紫外線照射に対し正常細胞と同レベルまで抵抗性を示す．3) A 因子がその機能を最大限発現するには，細胞質に注入後 2 時間を要し，それ以降約 14 時間の半減期で失活する．4) ゲル沪過により A 活性は分子量 16 万と 9 万の二つの画分に見いだされ，これらの画分は A 群から H 群までの XP 細胞のうち A 群に対してのみ有効である．

I-53. Antley Bixler Syndrome in 3 Japanese Children of 2 Families: **Kazuso IINUMA** (Natl. Child. Med. Res. Cent., Tokyo), **Tsutomu IWAYA** (Dept. Orthop., Jichi Med. Coll., Tochigi), **Takashi NAKAMURA** (Shizuoka Child. Hosp., Shizuoka), **Hiroyuki CHO and Yoshikazu KUROKI** (Kanagawa Child. Med. Cent., Kanagawa)

Three children featured by clinical resemblance with those previously described as a syndrome of multisynostotic osteodysgenesis (Antley Bixler syndrome) were demonstrated. A boy and a girl born to unrelated parents showed similar peculiar facies with minor deformities of ears, hands and feet. Both were suffering from ankylosis of elbow joints, though X-ray studies failed to disclose obvious osseous fusion (radio-humeral synostosis). Capitate and hamate were fused and bone ages were accelerated in both of them. The phenotypically normal father had a fusion of trapezoid and trapezium. The sister had labial fusion with large clitoris. Palpation suggested hypoplastic or aplastic uterus. The sibs had a mild degree of hearing loss. The brother later developed mild bronchitis, which resulted in unexpected death in a short time. A girl from another family was also noticed to share similar clinical features, including genital anomalies. She had also a fusion of capitate and hamate and accelerated ossification. All these patients were known of unusually rapid deterioration in respiratory functions when they got upper respiratory infections of mild type. Comparative studies with the literature point out that a wide clinical spectrum may characterize Antley Bixler syndrome.

I-54. The Clinical Course of the 3 Infantile Niikawa-Kuroki Syndrome: The Importance of Hypoglycemia, Elevation of Direct Bilirubin with Anemia in Neonatal Period: **Ryuichi TSUKINO, Syogo KIHIRA, Eizo HIRAISHI and Michio KOIKE** (Dept. Pediatr., Wakayama Med. Coll., Wakayama)

Observation of the clinical course of 3 cases of Niikawa-Kuroki syndrome revealed that in 2 out of 3 there were severe hypoglycemia, obstructive jaundice in neonatal period and normocytic anemia in second month. Case 1 and 2 were males delivered of our hospital. Their pregnancies were complicated by episode of an early rupture of the fetal membranes and cloudy amniotic fluid. Asphyxia (case 1) and convulsion (case 2) were noted soon after birth and both required intensive care. Blood glucose decreased as low as 26 mg/dl, 0 mg/dl in case 1 and 2, respectively and repeated glucose and glucagon infusions were needed especially in case 1. Blood chemistry revealed obstructuve jaundice, a total bilirubine of 11.7 mg/dl (direct Bil. 7.4 mg/dl) at 34 days after birth, 15.3 mg/dl (direct Bil.. 3.6 mg/dl) at 11 in case 1 and 2, respectively. Liver biopsy (case 1) at 34 days after birth demonstrated a neonatal hepatitis with marked hematopoiesis. Hematological examina-

tions showed normocytic anemia at 30–45 days after bith. Tube feeding was needed because of poor sucking from 1 to 6 months in case 3 (female), though details were not known.

I-55. Fetal Alcohol Syndrome: Analysis of Five Families: **Masato TSUKAHARA, Tsuyako EGUCHI, Tadashi KAJII** (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube), **Kazuaki YAMADA and Akio ASAKA** (Sch. Health Sci., Univ. Tokyo, Tokyo)

Five girls with fetal alcohol syndrome, born to a chronic alcoholic, two occupational drinkers and two housewives, were reported. Their major clinical features included pre- and postnatal growth retardation, mental retardation, blepharophimosis, ptosis, strabismus, microphthalmia, epicanthal folds, midface hypoplasia, hypoplastic ears, upturned nostrils, thin upper lip, cleft lip and palate, high arched palate, prominent labial frenum, short neck, labial hypoplasia, and abnormal dermatoglyphic patterns. Congenital heart defect was present in four patients. Agenesis of the right thumb and alternate hypertrophy were observed in a patient, suggesting that ethanol exerted its effect during organogenesis. An interstitial deletion of chromosome 13, 46,XX,del(13)(q21.2→q32), was present in a patient. The del(13) chromosome, being maternal in origin, was assumed to be the consequence of ethanol exposure during maternal meiosis. The phenotype of mitochondria aldehyde dehydrogenase (ALDH2), an isozyme that metabolizes aldehyde to acetic acid and is missing in 50% of the Japanese, was determined using hair roots in four patients (including the one with del 13) and their mothers. All mothers were ALDH2 positive, while all but one (a 21-year-old woman) of their offsprings studied were ALDH2 negative. The latter finding may be attributable to the immaturity of ALDH2 production in childhood.

I-56. Fetal alcohol syndrome (FAS) の母親の飲酒歴とアルデヒド脱水素酵素：山田一朗・浅香昭雄（東大・医・保健），**塚原正人・江口つや子・梶井　正**（山口大・医・小児）. **Five Mothers of Patients with Fetal Alcohol Syndrome (FAS) from the Viewpoint of Their Drinking Behavior and Aldehyde Dehydrogenase:** Kazuaki YAMADA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo), Masato TSUKAHARA, Tsuyako EGUCHI and Tadashi KAJII (Dept. Pediatr., Univ. Yamaguchi, Ube)

Mitochondrial aldehyde dehydrogenase (ALDH2) の多型現象と，個体の飲酒行動との密接な関連が注目されている．今回，5 例の FAS の母親について，ALDH2 の表現型同定を行ったところ，すべての母親が活性保有例，すなわち大量飲酒に陥りやすい遺伝的背景を有していた．またそのうち 2 例については，飲酒歴等の面接調査も合わせて行った．飲酒歴：(母親 1) 昭和 16 年生まれ，初回飲酒は 20 歳ごろであった．高卒後，事務職に従事した．29 歳で結婚し，36 歳から夫とともにスナ

ックを経営している．当初はごく薄い水割りを3～4杯飲む程度であったが，38歳のとき，夫の浮気を契機としてダブル～トリプルを5～6杯一気にあおる日が続いた．この時期が患児の妊娠初期であった．(母親2) 昭和25年生まれ．初回飲酒は18歳ごろ．高卒後，印刷業に勤めた後，21歳で結婚したが長女出産後間もなく離婚した．28歳から，スナックのホステスとなり，そこで知り合った2人の男性の間に次女，3女 (FAS患児) をもうけた．この間，ビールを中心に大びん6～8本を常飲していたというが，次女にはFASの症状はほとんど見られていない．両例とも職業的飲酒者ではあったが，アルコール依存の形成は見られていない．また，いずれも胎児へのアルコールの害についてほとんど無知であった．飲酒年齢の低年齢化，女性の飲酒者の急増が目立つ今日，飲酒教育をも含めた早急な対応が望まれる．

I-57. 教室における遺伝相談症例の検討：佐藤孝道・千谷東海・野末　順・香山文美・水野正彦 (東大・医・産婦人)．**Genetic Counselling at Tokyo University Hospital, Dept. of OB/GYN: Review of 1,051 Cases:** Kodo SATO, Tokai CHITANI, Jun NOZUE, Fumiyoshi KAYAMA and MASAHIKO MIZUNO (Dept. Obstet. Gynecol., Univ. Tokyo, Tokyo)

教室では昭和51年に遺伝外来を開設したが，以来59年末までの受診者数は1,051例に達した．受診者数は年々増加しており，最近は年間200例を超えている．1,051例は発端者別にみると，児に異常のあるものが53%でもっとも多く，クライアント本人または配偶者の異常は7%，その他の近親者の異常は9%であった．また，高齢妊娠は20%，反復流死産歴は16%であった．一方，疾患別にみると染色体異常が41%ともっとも多かったが，奇形12%，精神・神経疾患5%など疾患の種類は多岐に及んでいた．遺伝相談を行った症例のうち，553妊娠について出産児の予後を追跡しえたが，危険率2%未満と推定した393妊娠中6例 (1.5%)，2～9%と推定した76例中5例 (6.6%)，10%以上と推定した84例中24例 (28.6%) に該当疾患の患児が出産した．遺伝相談の内容別にみると，標準型21トリソミー児出産既往の228例の予後が判明したが，うち4例 (1.8%) は再びトリソミー児を妊娠していた (いずれも胎児診断を受け希望により中絶している)．奇形児出産既往の場合はメンデル遺伝も少なくなく胎児診断の意義は大きいが，主として超音波断層法による胎児診断を施行した47例中3例は患児であった．残り44例は正常と診断したが，うち1例は出産後前回児と同様脊椎裂であることが明らかとなった．以上，産科における遺伝相談は挙児の断念ではなく健康な児を持たせることを目的にしており，胎児診断を含め診断技術の開発，進歩の重要性が示唆された．

I-58. 突然変異率，浸透度および出生前淘汰を考慮した遺伝的危険率の推定について：吉丸博志・古庄敏行 (杏林大・保健・疫学)．**Estimation of the Genetic Risk Considering Mutation Rate, Penetrance and Prenatal Selection:** H. YOSHIMARU and T. FURUSHO (Dept. Epid., Kyorin Univ. Sch. Health Sci., Hachioji)

遺伝相談においては，遺伝的危険率の算出が一つの基礎となる．確率的な危険率の推定にはメンデル遺伝の考え方が基本となるが，著者らはさらに突然変異率 (μ)，浸透度 (ν)，出生前淘汰 (s′)，遺伝子頻度 (優性 p，劣性 q) などの遺伝的パラメータを考慮して危険率を推定する方法を考案し，こ

れに基づいた計算機プログラムを作成した．このプログラムは基本的な 12 種類の家系図に対して作成されており，汎用ではないが，サブルーチンプログラムの組み合せにより拡張が可能である．遺伝様式は，常染色体性の完全優性あるいは完全劣性を仮定した．計算例として，優性で $\mu=10^{-5}$, $\nu=0.7\sim1.0$, $s'=0\sim0.1$, $p=2\times10^{-5}$, 劣性で $\mu=10^{-5}$, $\nu=0.7\sim1.0$, $s'=0\sim0.1$, $q=4.5\times10^{-3}$ を仮定して危険率を算出し，これらの遺伝的パラメータ（とくに ν と s'）の影響を検討した．さまざまなパラメータが危険率の推定に影響を及ぼし，またパラメータの値も地域，性，年齢などにより異なることが考えられるので，あまり単純化しない，なるべく現実に即した推定法，およびきめ細かいパラメータの測定値がさらに追求される必要があろう．著者らは，さらに伴性遺伝の場合について同様に検討中である．

I-59. Consensus Survey on Heredity and the Handicapped (II): Norio FUJIKI, Masao NAKANAGA, Yoshiyuki OHNISHI (Dept. Intern. Med., Fukui Med. Sch., Fukui) **and Yasuko SHIRAI** (Dept. Soc. Welf., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Two years ago we reported that the younger, more educated, male, and longer profession with the handicapped care were ideally sympathetic to the handicapped and that the clients and persons working for the handicapped showed much better understanding concerning with practical problems. This meant the limited knowledge of heredity and the handicapped might lead to misunderstanding and prejudice and less recognition for these problems. This time we report the results of consensus survey regarding the prenatal diagnosis and selective abortion of high risk fetus among 400 medical staffs and 500 paramedical staffs, comparing with that of medical students, clients and general public in Fukui area. It revealed that they had attention toward the right to life of defective fetus as following orders; among general public, nurse student, pregnant mother with defective child, medical student, non-medical student, unmarried woman and mother with normal child; and among medical profession, public health nurse, pediatrician, midwife, gynecologist, internist. The results of consensus survey on these items and biotechnology among 600 persons in Fukui area revealed much improvement, though there still existed prejudice and misunderstanding on heredity. Finally we emphasize that more time for medical curriculum for human genetics and bioethics are necessary to educate general public.

I-60. Inheritance of Reciprocal Translocation Chromosomes in Man (1): Hidetsune OISHI[1] and Tsutomu YAMANAKA[2] ([1]Dept. Genet., Inst. Develop. Res., [2]Cent. Hosp., Aichi Prefect. Colony, Kasugai)

Since it was suggested that each chromosome arms were consisted of several functional units and that the break points by chromosome rearrangements with balanced conditions

were corresponded to the terminal ends of the units (1973), it is considered that the translocated chromosomes in the balanced condition are easily inherited to ones without any difficulty of the life in the following generations, and the inheritance of these chromosomes, as normal ones, is not selective during the course of reproduction. In 423 families examined for reciprocal translocation of chromosomes, male and female probands were 215 and 199, respectively, whereas total numbers of balanced carriers with apparently normal phenotype found in these families were 452 males and 652 females. By the pedigree analyses 132 males and 255 females with the same conditions of chromosomes were ascertained as the initial balanced carriers. In addition, balanced carriers were found in 260 of fathers and 475 of mothers, while numbers of male/female children in normal, balanced, and unbalanced conditions were 176/195, 321/394 and 344/314, respectively. Average number of spontaneous abortions in balanced carriers of female was also higher than that in these of males. These differences on parental numbers by sex were briefly discussed.

II-1. Polymorphism of EsD by Isoelectric Focusing: Evidence for the Existence of a New Allele: Nori KOMATSU, Masakazu OYA and Akira KIDO (Dept. Legal Med., Yamanashi Med. Univ., Yamanashi)

The polymorphism of EsD was analyzed on 1,115 individuals of Yamanashi Prefecture by the isoelectric focusing method developed by Yuasa *et al.* Besides the three common phenotypes, two heterozygotes, EsD 7-1 and EsD 7-2 were observed. The gene frequencies were: $EsD^*1=0.6234$, $EsD^*2=0.3663$ and $EsD^*7=0.0103$. During the course of our population survey we found an unusual isoenzyme pattern in the blood sample from a 56-aged man. The variant phenotype was characterized by three pairs of isoenzyme bands, one pair of which corresponded with the EsD 1 isoenzyme in electrophoretic mobility and the other two pairs migrated towards the anode. The banding profile of this variant resembles that of the rare EsD 6-1 type, but these two types are distinctly different in that the intermediate double bands of our variant move far more anodally than the EsD 2 isoenzyme while the middle band of the EsD 6-1 type is located more cathodally. The results of the family analysis strongly suggest the hereditary occurrence of a new variant allele at the EsD locus. We propose to designate tentatively this allele as *EsD*Kofu.*

II-2. Purification and Characterization of Esterase D 1 and D 2 from Human Erythrocytes: Evidence that They Are Monomers: Kiyosato MATSUO, Kunihiko KOBAYASHI, Keiji HAGIWARA and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Esterase D 1 and esterase D 2, two common esterase D [EC 3.1.1.1] isozymes, were isolated and purified from human erythrocytes. Their substrate specificity, pH profile and K_m values were essentially identical. Their molecular weight was the same at 34 k daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis and at 27 k daltons on Sephadex G-100 gel filtration. Antisera to each of the esterase D 1 and esterase D 2 isozymes were successfully raised in chickens. Each of them reacted identically with both isozymes. These findings indicate that the isozymes are close to each other in structure. The fact that the molecular weight of the esterase D 1 and esterase D 2 isozymes computed on sodium dodecyl sulfate polyacrylamide electrophoresis was close to that obtained on Sephadex G-100 gel filtration in non-dissociating buffer indicates that the isozymes are not dimers bound by disulfide bonds or a non-covalent force. These facts together indicate that the esterase D isozymes are monomers, contrary to the prevailing view that they are dimers (*Eur. J. Biochem.* in press).

II-3. Purification and Characterization of LCP1: Amino Acid Composition, Partial Amino Acid Sequences and Cell Distribution: **Kimiko YAMAKAWA, Ikuko KONDO, Hideo HAMAGUCHI** (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki), **Teizo KABASHIMA, Rikiya TSUNODA** (Inst. Clin. Med., Univ. Tsukuba, Ibaraki) **and Hideaki TANAKA** (Natl. Chem. Ind., Tsukuba, Ibaraki)

Lymphocyte cytosol polypeptide with molecular weight of 64,000 (LCP1: Mckusick #15343) is a human polymorphic polypeptide. The gene locus for LCP1 is closely linked to the EsD gene (Kondo & Hamaguchi, *Am. J. Hum. Genet.*, in press). This finding suggests that the locus for LCP1 is closely linked to the gene for retinoblastoma and Wilson disease. We purified LCP1 from B-lymphoblastoid cells and determined amino acid composition. Furthermore, we have examined cell and tissue distributions of LCP1 with immunohistochemical methods. The examined cells and tissues included peripheral blood lymphocytes, monocytes, granulocytes, cultured fibroblasts, cerebrum, heart, liver, kidney, stomach, small intestine, large intestine, lung, thymus, spleen, lymphnode, bone marrow, tonsil and adrenal grand. LCP1 was detected only in lymphocytes, granulocytes, monocytes and macrophages, but not in parenchymal cells of major tissues. Since the heterozygosity for LCP1 is rather low (12%) (Hamaguchi *et al.* 1982, *Hum. Genet.*) and since the expression of LCP1 is limited to specific kinds of cells, the establishment of the method to detect RFLPs of LCP1 seems to be useful for the genetic analysis of retinoblastoma and Wilson disease. We determined the amino acid sequences in the two parts of LCP1 (-Tyr-Ala-Phe-Val-Asn-, -Trp-Ala-Asn-Tyr-). The DNA probes corresponding to these amino acid sequences were synthesized. We set about the cloning of LCP1 gene.

II-4. Glutathion *S*-Transferase Polymorphism in Japanese and Its Pharmacogenetic Significance: **Shoji HARADA,[1] Masato ABEI[2] and Naomi TANAKA[2]** ([1]Inst. Comm. Med., [2]Inst. Clin. Med., Univ. Tsukuba, Ibaraki)

Human liver glutathion *S*-transferase (GST) catalyzes the conjugation of reduced glutathion (GSH) with various hydropholic substrates (Chausseand, 1979) and it is assumed that it plays a protective role against the effects of carcinogens, toxic chemical reagents and xenobiotics (Sparnis *et al.*, 1982). Recently, Board (1981) and Strange *et al.* (1984) reported that human GST isozymes are controlled by three different loci: GST_1, GST_2 and GST_3. They suggested that the isozymes at the GST_1 locus show highly polymorphic, which are classified as GST_11, GST_12–1, GST_12 and GST_10. In the present study, autopsy liver extracts from Japanese were examined for GST_1 locus phenotypes using starch gel electrophoresis in a Tris-malate-$MgCl_2$-EDTA/NaOH buffer system. Phenotypic distribution and gene frequencies are reported. Each isozyme from different GST loci was purified and was characterized on several biochemical properties. Moreover, the incidence of GST_1 null allele was investigated on the liver biopsy samples of the patients suffered from various liver diseases (liver hepatitis, fatty liver, liver cirrosis and hepatoma) from view points of etiology with GST_1 null type using polyacrylamide gel isoelectric focusing. The gene frequencies of the GST_11, GST_12, and GST_10 in Japanese were 0.252, 0.057, and 0.691, respectively. GST_10 was also detected as rare variant allele. The apparent K_m values of different isozymes from GST_1, GST_2, GST_3, and GST_4 were 604, 1,345, 776, and 591 μM for CDNB as substrate. The K_m values from these isozymes were distributed from 40 to 80 μM for GSH. The phenotype of GST_1 null was found in liver hepatitis and hepatoma as high frequencies (76.5 and 100%). However, normal liver and fatty liver showed the same frequency as mean value of Japanese population (50%). Therefore, it may be estimated that the individual possessing a common null allele is likely to occur in hepatic diseases due to the risk exposed to elevated level of certain electrophilic carcinogens. The more intensive studies are now undergoing.

II-5. GPT Polymorphism Detected in Human Urine by Isoelectric Focusing: **Koichiro KISHI, Toshihiro YASUDA and Reiko IIDA** (Dept. Legal Med., Fukui Med. Sch., Fukui)

Glutamate-pyruvate transaminase (GPT) polymorphism in human erythrocyte has been recognized, and three major phenotypes, GPT1, GPT2A, and GPT2A-1, were reported to be determined by two common alleles, GPT^1 and GPT^{2A}. In this study, we at first elucidated human urinary GPT polymorphism by isoelectric focusing in flat bed polyacrylamide gel. In the case of urinary GPT, isozyme patterns of homozygotes (GPT1 and GPT2A) showed 2 bands of activity, while heterozygote (GPT2A-1) gave 6 bands. This

urinary GPT isozyme profiles were very similar to the erythrocyte ones. Furthermore, the mode of inheritance of urinary GPT phenotypes was analyzed in 15 families with 29 children. These date indicate that polymorphism of urinary GPT can be explained by the existence of two common alleles, GPT^1 and GPT^{2A}. The estimated frequencies of GPT^1 and GPT^{2A} alleles calculated from urine samples of 205 unrelated individuals were 0.632 and 0.368, respectively ($\chi^2=0.304$, 1 df, $0.70>p>0.50$). When paired erythrocyte and urine samples from the same subjects were analyzed, it was found that GPT phenotyping of 120 subjects obtained from the urine sample agreed with the results from erythrocytes. From these results, urine was found to be a useful and valuable sample for the GPT phenotyping in the field of human genetics.

II-6. 日本人における核内 DNA の多型：植田信太郎（東大・理・人類）**・本庶 佑**（京大・医・医化）. **DNA Polymorphism in Japanese:** S. UEDA (Dept. Anthropol., Univ. Tokyo, Tokyo) and T. Honjo (Dept. Med. Chem., Univ. Kyoto, Kyoto)

ヒトの遺伝的多型現象の研究はこれまでタンパクを中心に研究が進められてきたが，サザン・ハイブリダイゼーション法により核内 DNA の多型を簡便に解析することが可能となった．最近，種々の遺伝性疾患に関し DNA 多型を用いた研究が行われているが，日本人一般集団における核内 DNA の多型の検索はいまだ十分に行われていない．今回われわれは，基礎的データを得るための手始めとして，免疫グロブリン C_ε 遺伝子に関する DNA 多型の検索を行った．$C_\varepsilon 1$ 遺伝子では，制限酵素 *Bam*HI および *Eco*RI を用いたとき DNA 多型がみられ，とくに *Eco*RI では高頻度で観察された．また現在の $C_\varepsilon 2$ 遺伝子は祖先型 $C_\varepsilon 1$ 遺伝子が 660～890 万年前に重複を起こし，さらに欠失を生じたことにより形成されたと考えられる．この欠失は現在のすべての $C_\varepsilon 2$ 遺伝子に存在するのか否かを検索したが，非欠失型 $C_\varepsilon 2$ 遺伝子は 1 例も観察されなかった．ヒトと共通の祖先型 $C_\varepsilon 2$ 遺伝子をもつアフリカ産類人猿でも類似の欠失が生じており，祖先型 $C_\varepsilon 2$ 遺伝子は偽遺伝子化して集団中に固定していると推測された．

II-7. Mitochondrial DNA Polymorphism in Japanese Living in Hokkaido: Shinji HARIHARA, Momoki HIRAI and Keiichi OMOTO (Dept. Anthropol., Fac. Sci., Univ. Tokyo, Tokyo)

Restriction enzyme fragment patterns of human mitochondrial DNA (mtDNA) were analyzed using total DNAs extracted from blood cells of 122 Japanese (48 Ainu and 74 Non-Ainu) living in Hokkaido. Variations were detected using the Southern hybridization method: Six *Ava*II morphs, four *Hinc*II morphs, three *Hpa*I morphs and two *Pvu*II morphs were found. Among them, the following three morphs, which were found in the Non-Ainu, have not been reported previously: *Ava*II morph 12, *Ava*II morph 13 and *Pvu*II morph 3. In *Ava*II morph 12, an extra site is generated due to the substitution (G to A transition

or G to T transversion) at bp 6,384 and a site loss is located at bp 16,269. In *Ava*II morph 13, a site loss occurs at bp 12,629. In *Pvu*II morph 3, the base substitution at bp 12,753 (A to G transition) generates an extra *Pvu*II site. By combining the results of the restriction patterns, the mtDNAs of the Japanese were classified into eleven types. Though the mtDNAs of the Ainu Japanese were less polymorphic than those of the Non-Ainu, the distribution of mtDNA morphs was not significantly different between the Ainu and Non-Ainu Japanese.

II-8. Mitochondrial DNA Polymorphism in Japanese. II. Analysis with Restriction Enzymes of Four or Five Base Pair Recognition: Satoshi HORAI and Ei MATSUNAGA (Dept. Hum. Genet., Natl. Inst. Genet., Mishima)

Mitochondrial DNA (mtDNA) from 116 Japanese were analyzed with 9 restriction enzymes that recognize a four or five base sequence. The size of the mtDNA fragments produced by digestion by each enzyme were compared after gel electrophoresis. Double digestion experiments indicated that, in the coding region from URF2 to $tRNA^{Asn}$ (bp 5,274–5,691), there is an insertion of about 60 base pairs compared with the published mtDNA sequence, which is common to all individuals in the present sample. A total of 95 different morphs were detected with the 9 enzymes, 60 of which have not been documented previously. The high frequency of new polymorphisms is, however, due to the fact that our survey is the first extensive analysis of mtDNA polymorphism in the Japanese population. Although Cann (1982) also analyzed mtDNA from many Orientals, only two Japanese were included. Therefore, this finding may reflect a unique profile of the Japanese population inferred from mtDNA polymorphisms. This is supported by our previous analysis with enzymes of hexanucleotide recognition, where several new polymorphisms with various enzymes were detected. Based on a comparison of the cleavage maps of all individuals, 62 different combinations of restriction types were observed. By pairwise comparison of each restriction type, the average number of nucleotide substitutions per nucleotide site (δ) was estimated to be 0.0026. Phylogenetic analysis of the present data indicates that at least two distinct lineages exist in the Japanese population.

II-9. Expression and Distribution of Human Blood Group Antigens in Nonhuman Primates: Tamiko NAKAJIMA, Seiko MIYAZAKI, Tadahisa KOGURE and Ken FURUKAWA (Dept. Legal Med., Sch. Med., Gunma Univ., Maebashi)

Group A chimpanzees, AB orangutan, and B and AB gibbons were determined by hemagglutination with monoclonal anti-A and anti-B antibody. Distribution of group AB prosimians, B and AB new world monkeys, and O, A, and B old world monkeys were

demonstrated with elution test of human anti-A and anti-B sera. The results of the red cell typing can be confirmed by testing their serum for the presence of anti-A and/or anti-B agglutinins, except in new world monkeys. The O and B type Japanese monkeys can still be established from the presence of the H and B substances in the tissue extract of stomach, salivary gland and upper part of small intestine. Measurements of the *N*-acetylgalactosaminyltransferase (A enzyme) and galactosyltransferase (B enzyme) activities in sera of nonhuman primates were carried out by enzymatic conversion of human group O red cells into A and B active cells. The A and B transferase activities of the nonhuman primates were not much weaker than that of human group A_1 and B subjects. M, P, Le^a and Le^b antigens were weakly expressed and distributed in the old world monkeys and apes. Expression of c(hr′), D(Rh_o), k, Fy^b, Lu^b, and Jk^a antigens were detected in gibbons, orangutan and chimpanzees by absorption and elution tests.

II-10. Molecular Basis of Structure and Expression of DQw1 β Gene from HLA-Dw2 and Dw12: **Kikuo TSUKAMOTO** (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Michio YASUNAMI, Kazuhiko FUJISAWA and Takehiko SASAZUKI** (Dept. Genet., Med. Inst. Bioregul., Kyushu Univ., Fukuoka)

HLA class II molecules, DR, DQ and DP, are encoded within HLA-D region which controls the immune response, disease susceptibility and both primary and secondary MLR. HLA-linked immune suppression genes (Is-gene) which control the low or non responsiveness through antigen specific suppressor T cells are also mapped within HLA-D region. HLA-Dw2 and Dw12 show remarkable difference in their properties of immune response to several antigens and in their disease susceptibility. Functional analysis of class II molecules from these two haplotypes revealed that a) at least two DR, one DQ and one DP molecules are expressed on Dw2 or Dw12 homozygous cells, b) DR molecules act as a product of HLA-linked Ir-gene and provoke primary MLR, c) the DP molecule provokes secondary MLR and d) the DQ molecule is the best candidate for the product of HLA-linked Is-gene since only anti-DQ monoclonal antibody can restore the responsiveness of nonresponder to those natural antigens. Analysis of structure and expression of DQw1 β genes demonstrates that the DQw1 β gene from Dw12 consist of 6 exons and express multiple forms of β polypeptide, which may be caused by altered RNA splicing. The predominant product of DQw1 β gene of Dw12 consist of 237 amino acids, whereas Dw2 cells express only one β polypeptide consist of 229 amino acids since DQw1 β gene of Dw2 would be consist of 5 exons like the other DQ β genes which lacks a cytoplasmic exon corresponding to exon-5 of DQw1 β gene of Dw12. They are different in their amino acid sequence of β1 domain, which would be recognized by alloreactive or antigen specific T

cell receptors. The anomalous structure of DQw1 β of Dw12 which resembles that of murine I-A β suggests that the DQw1 molecules of Dw12 may play important role in control of immune response as I-A molecule does.

II-11. Alloreactive T Cell Clones which Detect the Polymorphic Site of HLA-DR Molecules ($\alpha\beta$1 and $\alpha\beta$2 Chains) of HLA-Dw12 Haplotype: **Kenji HIRAYAMA, Yasuharu NISHIMURA, Mitsuru FUKUNAGA and Takehiko SASAZUKI** (Dept. Genet., Med. Inst. Bioregul., Kyushu Univ., Fukuoka)

HLA-class II molecules encoded by the genes in the HLA-D region have been reported to control immune responsiveness and disease susceptibility in man. The heterogeneity of host-reaction in response to foreign antigens or pathogens should be generated by the polymorphism of the HLA-class II molecules. The polymorphism of class II molecules are detected by immunocompetent T cells to provoke various immunological responses. To analyze the polymorphism and function of these HLA-class II molecules, we have established alloreactive T cell clones which recognized class II molecules of HLA-Dw12 haplotype by the method of limiting dilution. T cell clone MF-25 reacted to Dw12 positive panels and did not show any response to Dw12 negative panels. This clone detected the $\alpha\beta$2 chain of DR molecule of Dw12 haplotype. T cell clone HR-42 reacted to DR2 positive panels and did not show any proliferative response to DR2 negative panels. The response of HR-42 was completely inhibited by both anti OR framework monoclonal antibody and anti DR2 monoclonal antibody. Therefore, this T cell clone which detected the polymorphic site of DR($\alpha\beta$1) molecules of Dw12 haplotype were shown to be recognized by alloreactive T cells. Moreover, these DR molecules acted as restriction molecules of the cooperation between soluble antigen specific T cell clone and monocytes. From all these data, we concluded that both DR molecules play important roles in immune system.

II-12. A Human Cytotoxic T Cell Clone Recognizing a Hybrid DQ Antigen: **Yukio KAKUTA, Yuichiro FUKASAWA, Shuichi HAWKIN, Hiroshi KOJIMA, Akemi WAKISAKA and Miki AIZAWA** (1st Dept. Pathol., Hokkaido Univ. Med. Sch., Sapporo)

Recent studies using two-dimensional gel electrophoresis suggest that hybrid DQ molecules are expressed in cells heterozygous for HLA-D region. In mice, the functional role of hybrid I-A antigens in immune responses is well established. In human, however, the functional significance of hybrid DQ molecules remains unknown. In the present study, alloreactive cytotoxic T cell (CTL) clones generated against a heterozygous B cell line, EBV-Fuk (A24, B7, Cw7, DR1, DQw1, Dw1/A24, Bw54, Cw1, DR4, DQWa, Dw15)

were analyzed. The CTL clone 5-44 reacted with EBV-Fuk and EBV-Wak whose HLA-haplotypes were entirely identical, whereas 5-44 did not react with homozygous B cell lines, EBV-Sa (A24, B7, Cw7, DR1, DQw1, Dw1) and EBV-Wa (A24, Bw54, Cw1, DR4, DQWa, Dw15). Moreover, the cytolytic activity of 5-44 was inhibited by an anti-DQWa monoclonal antibody (MoAb), HU-46, but it was not affected by either an anti-DQw1 MoAb, HU-11, or anti-DR MoAbs, HU-4 and HU-20. As the Western blotting analysis showed that HU-11 recognized a determinant borne on the beta chain of DQw1 antigens, the CTL clone 5-44 was thought to recognize the hybrid DQ molecule formed by the alpha chain of DQw1 and the beta chain of DQWa. This indicates that a novel determinant created by the trans-association of DQ alpha and DQ beta chains can be recognized by CTL. In human, hybrid DQ antigens are assumed to contribute to the increase of the repertoire of HLA-D region products and to play an important role in immune responses.

II-13. A Monoclonal Antibody that Detects a Public Specificity of HLA-A Locus Antigens: **Yuichiro FUKASAWA, Yasutaka TAJIMA, Hiroshi KUNIKANE Hiroshi KOJIMA, Tsuguyo NAKAYAMA, Kazumasa OGASAWARA, Akemi WAKISAKA and Miki AIZAWA** (1st Dept. Pathol., Hokkaido Univ. Med. Sch., Sapporo)

In order to study the gene products of HLA complex, a monoclonal antibody, termed HU-34, was produced by immunizing BALB/c mice with a cultured human B lymphoblastoid cell line, RPMI 8057 (A26, 30; B7, 35; DR1, 4; Dw1, w4). When the serological specificity was evaluated with a panel of 635 healthy Japanese donors, it was found that HU-34 reacted with all but lymphocytes typed as "HLA-A24, blank," which were assumed to be homozygous for A24. Moreover, in two informative families, the reactivity of HU-34 was shown to segregate with HLA-A locus antigens. To make sure that HU-34 recognized a determinant borne on HLA-A locus antigens, "lysostrip" analysis was performed. When lymphocytes were pretreated with HU-34 and anti-mouse Ig $F(ab')_2$ fragments before the complement-dependent microcytotoxicity test, the reactivity of conventional antisera specific for HLA-A locus antigens except A9 (or A24) was reduced, but that of antisera specific for HLA-B and -C locus antigens was not affected. In addition, the reactivity of monoclonal antibodies recognizing A2 and A28 antigens was also inhibited by the same pretreatment. These results indicate that HU-34 recognizes a common determinant among HLA-A locus antigens but not borne on A24. Consequently, the existence of "A-ness" can be detected by use of monoclonal antibody, HU-34, recognizing a public specificity of HLA-A locus antigens, as "B-ness" is determined by the diallelic antigen system of Bw4 and Bw6.

II-14. (Not Presented)

II-15. Sarcoidosis and HLA Antigen: **Hiroshi KUNIKANE, Tsuguyo NAKAYAMA, Yasutaka TAJIMA, Akemi WAKISAKA and Miki AIZAWA** (Dept. Pathol., Hokkaido Univ. Sch. Med., Sapporo)

Sarcoidosis is a systemic granulomatous disease, and immune factors seem to take part in its pathogenesis. Many studies on the association of HLA antigens with sarcoidosis have been reported. However, there were few studies dealing with HLA-D region antigens and no clear conclusion about the association was established. In this study, 53 sarcoidosis patients, all of whom had a respiratory symptom as a chief complaint, were HLA typed. Sixty and 57 unrelated healthy panels served as controls for HLA-A, B, C and HLA-D region antigens, respectively. In HLA-D region antigens, the frequency of DRw52 significantly increased in the patients (79.2% as compared with 50.9% of the controls: $\chi^2=9.6$, $Pc<0.05$, R.R.$=3.7$), whereas no significant increase was noted for HLA-A, B, C antigens. In addition, when various clinical features were taken into account, 31 (96.6%) out of 32 patients without an ophthalmic involvement were DRw52-positive ($\chi^2=19.7$, $Pc<0.01$, R.R.$=29.9$, compared with the controls). By contrast, DRw52-positive were 11 out of 21 (52.4%) among the patients with an ophthalmic involvement, almost as much as among the controls. These results suggest that there may be a heterogeneity in the entity of sarcoidosis, as well as that HLA-D region antigen may be concerned in its pathogenesis.

II-16. Immunogenetic Analysis of Leprosy: **Ikuo KIKUCHI, Takehiko SASAZUKI** (Dept. Genet., Med. Inst. Bioregul., Kyushu Univ., Fukuoka), **Toshiharu OZAWA** (Natl. Inst. Leprosy Res., Tokyo), **Kiyotaka SANADA, Masanori KOSEKI** (Natl. Sanat. Tama-zenshoen, Tokyo), **Jun TANAKA, Akinori KOHZUMA and Shigeru KUMAMARU** (Natl. Sanat. Kikuchi-keifuen, Kumamoto)

Genetic control of the clinical manifestation of leprosy was investigated using 66 unrelated patients with leprosy. In the patients, HLA-DR2 and -DQw1 were significantly increased compared to healthy controls (relative risk$=3.76$, $\chi^2=16.60$, relative risk$=4.09$, $\chi^2=11.10$, respectively). In patients with lepromatous leprosy HLA-B35-DR2-Dw2 haplotype was significantly increased (relative risk$=9.23$, $\chi^2=11.78$), whereas in patients with tuberculoid leprosy the increase of HLA-B35-DR2-Dw2 haplotype was not significant. Affected sibpair method revealed that the major gene controlling the clinical feature of lepromatous leprosy was linked with HLA ($\chi^2=6.78$, D.F.$=2$) and that the phenotype controlled by the HLA-linked major gene for lepromatous leprosy might be a dominant trait. Using unrelated patients with lepromatous leprosy, it was confirmed that the phenotype controlled by the

HLA-linked major gene for lepromatous leprosy might be a dominant trait. Furthermore the presence of antigen specific suppressor T cells ($Leu2^{+}3^{-}$) was demonstrated in patients with lepromatous leprosy. All these observations suggested that the clinical manifestation of leprosy was controlled by HLA-linked immune suppression gene.

II-17. Study of Genetic Variation among the Japanese (in Hiroshima, Nagasaki) Using Two-Dimensional Electrophoresis. V. Proteins in Erythrocyte Lysates (Part 2): Yuko NAGAHATA-SHIMOICHI, Norio TAKAHASHI, Jun-ichi ASAKAWA and Yoshiko TANAKA (RERF, Hiroshima)

We have been engaged in the study of genetic variation of proteins in erythrocyte lysates using two-dimensional electrophoresis (2-D PAGE). The results of our studies obtained from 100 individuals were reported in part at the last Annual Meeting of this Society. Subsequently, improvements in the conditions of electrophoresis and staining have made it possible to score 15 polypeptides in addition to the 40 polypeptides previously studied. Therefore, a review was made on the above 100 individuals for genetic variation of these 55 polypeptides. Furthermore, a similar study was made on additional 70 individuals using 2-D PAGE. The results are presented together. Erythrocyte samples were obtained from participants in the previous RERF Biochemical Genetics Study. Modifications of the existing electrophoresis and staining methods[1] were employed. Among 55 kinds of polypeptides scored, genetic variation was observed in 15, including the four previously reported. Phenotypes of these 15 polypeptides were compared with those of 22 erythrocyte enzymes determined by starch gel electrophoresis in our laboratory, but no correlation could be demonstrated between the two groups except for esterase D which had already been identified on 2-D PAGE by Kondo *et al.*[2]

1) Takahashi, N. *et al.* 1985. *Jpn. J. Human Genet.* **30**: 98.
2) Kondo, I. *et al.* 1984. *Hum. Genet.* **66**: 244.

II-18. Study of Genetic Variation among the Japanese (in Hiroshima, Nagasaki) Using Two-Dimensional Electrophoresis. VI. Genetic Variants of Polypeptides in Erythrocyte Lysates: Norio TAKAHASHI, Yuko NAGAHATA-SHIMOICHI, Jun-ichi ASAKAWA and Yoshiko TANAKA (RERF, Hiroshima)

Genetic variation of polypeptides was studied in erythrocyte lysates from 170 Japanese and their parents by means of two-dimensional polyacrylamide gel electrophoresis. Genetic variants were observed in 15 kinds of polypeptides among 55 polypeptides selected for the examination. This presentation reports on the nature of variants encountered in the Japanese population. Among the 15 variant polypeptides, 12 showed different isoelectric points

from their normal spots and 5 of them also had altered molecular weights. In contrast, 2 of the 15 variants were found to have only altered molecular weights. A complete defect of a polypeptide named "D-30" was also observed in a male individual. The results of family studies (on parents, two brothers, and two sisters) suggested that this peptide is coded for by a gene located on the X-chromosome. The molecular weight of this peptide was therefore compared with those of polypeptides whose genes are known to be located on the X-chromosome, but no correspondence could be observed in their molecular weights. Among the 15 polypeptides in which variants were observed for Japanese, only 8 have been examined for Caucasians and no variant form has been detected for 3 of the polypeptides.[1] In addition, significant differences in frequencies were observed in 2 of the 5 polymorphic systems identified for both ethnic populations. Even though the numbers examined are still relatively small and all conclusions are tentative, ethnic differences appear to be emerging.

1) Rosenblum, B.B. *et al.* 1984. *Am. J. Hum. Genet.* **36**: 601.

II-19. Electrophoretic Variants of Haptoglobin Found in the Children of Atomic Bomb Survivors: Jun-ichi ASAKAWA, Mikio FUJITA, Chiyoko SATOH, Yuko NAGAHATA-SHIMOICHI, Yoshiko TANAKA, Satomichi KANEOKA, Kazuaki GORIKI (RERF, Hiroshima) **and Ryuji HAZAMA** (RERF, Nagasaki)

A total of 23,326 children of atomic bomb survivors in Hiroshima and Nagasaki were examined for plasma haptoglobin (HP) by starch gel electrophoresis: 12,865 children were born to proximally exposed parents and 10,461 were children of distally exposed parents selected as controls for the first group. Variants having abnormal mobility were encountered in 44 children. Genetic nature of variants were confirmed by family studies in all instances in which both parents could be examined except one case where a putative mutant was detected in a female Nagasaki child of the control group. These variants including the mutant were compared with respect to their relative mobilities using polyacrylamide slab gel electrophoresis and classified into 12 phenotypes including those similar to 1-Johnson (1-J) and 2-Johnson (2-J). The mutant showed abnormal phenotype consisted of a set of bands with a slightly faster mobility than the HP 2 bands. Both parents and 4 sisters of the propositus exhibited HP 2 phenotype with no abnormal bands. No parentage discrepancy was revealed by blood, protein and HLA typings except the HP type. These variants were purified by affinity chromatography and examined by O'Farrell's two-dimensional electrophoresis (2-D) in order to find out in which polypeptide chains of haptoglobin the variation occurred. Existence of 2 types of polypeptide for Johnson-like variants were observed when variant haptoglobins from plasma of individuals having phenotype similar

to 1-J or 2-J were examined by 2-D. Both of them seemed to have the same molecular weight (about 28 k dalton) but migrated to different positions in the isoelectric focusing axis. In addition, 3 types of β-chain variant were also observed.

II-20. Evaluation of the Genetic Effects of Radiation of Atomic Bombs at the Protein Level: Chiyoko SATOH, Kazuaki GORIKI, Jun-ichi ASAKAWA, Mikio FUJITA, Norio TAKAHASHI, H.B. HAMILTON (RERF, Hiroshima), **Ryuji HAZAMA** (RERF, Nagasaki) **and J.V. NEEL** (Univ. Michigan)

Studies to detect mutations at the protein level to evaluate genetic effects of radiation of atomic bombs have been carried out in Hiroshima and Nagasaki. Children of proximally exposed survivors and those of distally exposed survivors selected as controls were examined. Each child was examined for rare electrophoretic variants of 30 proteins of the blood plasma and erythrocytes. A subset of the children was further examined for "deficiency" variants of 11 erythrocyte enzymes. A rare electrophoretic variant is defined as a variant with a phenotype frequency of less than 1% in the population and "deficiency" variant as one with enzyme activity level $\leqslant 66\%$ of normal. When either type of variant was encountered, blood samples from both parents were examined. When neither parent exhibited the variant, a discrepancy between legal and biological parentage was explored with studies of blood, protein and HLA types. Using electrophoresis, 13,052 children of the proximally exposed parents and 10,609 control children were examined. The number of equivalent locus tests was estimated to be 725,587 for the exposed and 539,170 for the control group. Since 3 fresh mutants were observed in each group, mutation rates are 0.4×10^{-5} and 0.6×10^{-5} per locus per generation for the former and the latter, respectively. With enzyme activity measurement, the number of equivalent locus tests was 59,781 for the former and 62,180 for the latter. One deficiency mutant was encountered in the exposed, the mutation rate being 1.7×10^{-5} per locus per generation while no mutation was observed for the control. So far no measurable genetic effects due to A-bomb exposure of the parents has been observed.

II-21. Survey of Hemoglobinopathies in Aichi District: Hiromi KEINO, Koji SHIMIZU (Inst. Develop. Res., Aichi Pref. Colony, Aichi) **and Iwazo HASEGAWA** (Aichi Red Cross Blood Cent., Nagoya)

We surveyed 32,097 healthy blood donors at Aichi Red Cross Blood Center for the detection of hemoglobinopathies for about one year (1983–1984). We found eight α-globin, two β-globin and one δ-globin chain variant carriers as well as one δ-thalassemia homozygote and two δ-thalassemia heterozygotes with elevated Hb F. Structural analyses

using micro-sequencing method by Chang (1978) revealed the presence of one Hb Kokura (α47 Asp→Gly), one Hb Chad (α23 Glu→Lys), two Hb Queens (α34 Leu→Arg) which was first reported in Koreans, one Hb Hoshida (β43 Glu→Gln), and Hb Camden (β131 Gln→Glu) up to this time. Incidence of variant carriers in Aichi district was about 0.03% as usual among Japanese (Ueda, 1985), but 0.01% less than that of southern Aichi people (Ueda, 1985), and almost one tenth of that of Chinese (Zeng, 1985). Incidence of α-globin variant carrier was about four times as many as that of β-globin variant carriers as generally seen among Chinese and Japanese.

II-22. Abnormal Hemoglobins in Fukuyama District and Different Rates of Stable α-Globin Variant Chain: **Kazuo HIDAKA, Iwao IUCHI, Shunichi SHIMASAKI** (Kawasaki Med. Sch., Kurashiki) **and Goro IWAKAWA** (Natl. Fukuyama Hosp., Fukuyama)

A hemoglobinopathic survey in Fukuyama area was conducted in the individuals totaling 8,900 from April to October in 1985. Five fast-moving abnormal hemoglobins were detected and referred as α chain anomaly. All of five variants were confirmed as Hb Ube-2 (α68 Asn→Asp) by the analyses of HPLC of the tryptic digest of α chain, of fingerprint of the thermolysin digest of aberrant peptide and amino acid analysis. Sixteen carriers of Hb Ube-2 have already been discovered: Ube (one case), Kagawa (6), Okayama (1), Hyogo (5), Osaka (2) and Aichi (1). Therefore, these five variants were corresponded to 17–21 instances of Hb Ube-2. Hb Ube-2 may be classified as a sporadically distributed Hb among Japanese. Hb Ube-2 was shown to be expressed in heterozygotes and it's amounts were 16.4%, 20.0–20.8% and 22.0–24.8% of total hemoglobin.

We determined the presence of the third α-globin locus by restriction enzymatic digestion and hybridization with α-globin probe. Two cases who were heterozygous with five α-globin gene ($\alpha\alpha/\alpha\alpha\alpha$) were discovered from 104 individuals. Therefore, the detection rate of these heterozygote was one per 50 individuals with a frequency of 1.9%. When the presence of individuals with five α-globin gene ($\alpha\alpha/\alpha\alpha\alpha$) or six α-globin gene ($\alpha\alpha\alpha/\alpha\alpha\alpha$) is considered, an explanation for the low proportion of Hb Ube-2 (20.0%, 16.4%) may be provided. But a definitive explanation of the low proportion requires further study.

II-23. 鹿児島・沖縄地区における G6PD 異常症の頻度調査：鈴木早苗・高橋圭介・藤井寿一・三輪史朗（東大・医科研・病態薬理）**・尾辻省吾**（鹿児島大・中央検査）**・古波倉正照**（沖縄・古波倉内科医）. **Frequency of G6PD Deficiency in Kagoshima and Okinawa Area:** Sanae SUZUKI, Keisuke TAKAHASHI, Hisaichi FUJII, Shiro MIWA (Dept. Pathol. Pharmacol., Inst. Med. Sci., Univ. Tokyo, Tokyo),

Shogo OTSUJI (Dept. Cent. Lab., Kagoshima Univ., Sch. Med., Kagoshima) and Masateru KOBAKURA (Naha, Okinawa)

グルコース-6-リン酸脱水素酵素 (G6PD) 異常症は，世界でもっとも頻度が高い赤血球酵素異常症であり，多くは無症状だが薬剤惹起性溶血発作を起こすものもある．日本においては全国的な規模でのスクリーニングは行われていないが，われわれは山口県，東京地区に引き続いて，今回は鹿児島・沖縄地区においてスクリーニングを行った．われわれが開発したホルマザンリング法を用いた．これは G6PD の反応液を含む寒天上に，PKU 沪紙にしみこませた検体を置き，ホルマザンリングの大きさを測定する方法である．鹿児島地区では，3,003 名中 2 例（頻度 0.06%）の G6PD 異常症を発見した．沖縄地区では，本島 1,430 名，宮古島 377 名，八重山諸島 501 名行ったが，異常症はまだ発見されていない．また札幌地区も 597 名行ったが，異常症は見つかっていない．G6PD 異常症は東南アジア地方で頻度が高く，南の鹿児島・沖縄地区では日本でも頻度が高いのではないかと予想されたが，今回のスクリーニングの結果では，東京 (0.055%)，山口県地区 (0.2%) と比較して大差なく，南方から鹿児島・沖縄地区への G6PD 異常症の abnormal gene の流入の頻度は低いと考えられる．今後は，沖縄・札幌地区でさらにスクリーニングと変異酵素の同定を行い検討していく予定である．

II-24. The Distribution of Gc Subtypes in Japanese and the Neighboring Populations: Relations to the Geographical Cline in Japan: **Keiichi OMOTO** (Dept. Anthropol., Univ. Tokyo, Tokyo) **and Kyung Sook PARK** (Dept. Biol., Sungshin Womens Univ., Seoul)

Recently, the presence of geographical cline of the Gc subtype frequency in Japanese was reported (Yuasa *et al.*, 1983; Omoto, 1985). The allele frequency of *Gc*1F* tends to be slightly lower, while that of *Gc*2* higher in the western part than in the eastern part of Japan. The Ainu with an exceedingly high *Gc*1F* and a relatively low *Gc*2* frequency (Omoto and Miyake, 1979) appears to be on the eastern extreme in this respect. To shed further light on the question of the cline, population samples from Okinawa and Korea were examined with respect to Gc subtypes. In a sample of 521 sera from Ishigaki City, Okinawa, the allele frequencies were: *Gc*1F:* 0.4932, *Gc*1S:* 0.2095, *Gc*2:* 0.2934, *Gc*1A2:* 0.0039. Rare variant phenotypes were encountered eight times. In a sample of 220 sera from Seoul, Korea, the frequencies were: *Gc*1F:* 0.4413, *Gc*1S:* 0.2300, *Gc*2:* 0.3146, *Gc*1A2:* 0.0141. Seven cases of rare variants were detected. These results are consistent with the hypotheses that the Gc cline from western to eastern Japan is formed by gene flow, and that this cline is independent from that in the south-western part of Japan including Okinawa.

Omoto, K. 1985. *J. Anthrop. Soc. Nippon* **94**(1) in press.
Omoto, K. and Miyake, K. 1979. *Jpn. J. Human Genet.* **24**: 224.
Yuasa, I. *et al.* 1983. *Jpn. J. Human Genet.* **28**: 255.

II-25. Genetic Polymorphism of α_2HS Glycoprotein and Group-Specific Component in Egyptian: **S. ABE, K. HIRAIWA** (Dept. Legal Med., Fukushima Med., Coll., Fukushima) **and I.M. SEBETAN** (Dept. Forens. Med., Tohoku Univ., Sendai)

The distribution of α_2HS glycoprotein (AHS) and group-specific component (GC) were studied with isoelectric focusing method from 132 Egyptians serum samples in total. AHS, three common types, AHS 1, 2-1 and 2, were observed, and the frequencies of AHS alleles were estimated to be *AHS*1*=0.8579 and *AHS*2*=0.1421 (n=95). In Japanese population living in Fukushima, they were found to be *AHS*1*=0.7585 and *AHS*2*=0.2415 (n=706). *AHS*1* frequency in Egyptian is higher than Japanese (0.7325–0.7670, Umetsu 1984, Yuasa 1984), Nepalese (0.7571, Yuasa 1984), German (0.654, Weidinger 1984) and Canadian (0.640, Cox 1983) populations. As to GC, six common subtypes, 1F, 1S, 1F1S, 2-1F, 2-1S and 2, were identified. The present study revealed no variants. The allele frequencies for the genes *GC*1F*, *1S* and *2* were found to be 0.2538, 0.5265 and 0.2197, respectively (n=132). By immuno-electrophoresis, Goedde (1980) reported that the gene frequency of *GC*1* was estimated to be 0.834 (n=154), while this study showed it to be 0.780. The distribution of GC allele frequencies are similar to that of Arabian populations hitherto reported (Israel: *GC*1F*=0.2231, *1S*=0.5436, *2*=0.2308; Tunisian: *GC*1F*=0.260, *1S*=0.525, *2*=0.215).

II-26. Study of Consanguinity in Isolated Community, Miyama-cho (III): **Norio FUJIKI, Mikio HIRAYAMA, Motozumi NOMURA, Yoshiyuki OHNISHI, Masao NAKANAGA** (Dept. Intern. Med., Fukui Med. Sch., Fukui), **Masuji MORITA** (Dept. Public Health., Kyoto Pref. Univ. Med., Kyoto) **and Kazuo MANO** (Dept. Neurol., Nagoya Natl. Hosp., Nagoya)

Since 1982 we have made medical and genetic survey in two mountaneous communities (hamlets A and K) in Miyama-cho, Fukui Prefecture consisting of 271 households and 876 inhabitants. We have reported the breeding structure of these hamlets from the data basis transcribed from the microfilmed Koseki files. This time we could calculate the consanguinity rate and mean inbreeding coefficients for living couples and sibs. The consanguinity rate of hamlet K was higher than that of hamlet A as well as the endogamy rate, though the isolated breaking was abruptly occurring in these hamlets. Summarizingly these hamlets showed: 15.7% consanguinity rate with 6.6% 1C marriage, 0.0066 of mean inbreeding coefficient per living couple and 0.0059 of mean inbreeding coefficient per living sib, which corresponded in average slightly remote consanguinity of 2.5C marriage. As shown in these figures there existed still frequently high consanguinity, especially repetitive consanguinity like 1C+1C, 1C+1.5C, 1C+1.5C+2C even in younger generation in

hamlet K. On the other hand medical survey did not reveal increased occurrence of hereditary diseases or congenital malformation instead of high inbreeding.

II-27. Factors for Decline of Consanguineous Marriages: **Yoko IMAIZUMI** (Inst. Population Problems, Minist. Health Welfare, Tokyo)

A survey of consanguineous marriages in Japan was conducted on September 1, 1983 through questionnaires. The total number of couples studied was 9,225. They were chosen from six widely different areas of Japan. The rates of first cousin marriages and of total consanguineous marriages for all areas are 1.6% and 3.9%, respectively. These rates are decreased with marriage year in Japan. The rates of consanguineous marriage are influenced by last school completed, occupation, religion, marital distance between birthplaces, surname (concordance or discordance), marriage form (love match or arranged marriages), circumstances of encounter, and marital motivation. The declining rates of consanguineous marriage are positively correlated with the rates for graduates of junior high school, agriculture, Buddhists, the same birthplace between spouses, surname concordance, arranged marriage, childhood fried, and external pressure towards marital motivation.

II-28. DNA 多型と父親の排除確率：伊藤綽子・安田徳一（放医研・遺伝），**松本秀雄**（大阪医大・法医）. **The Probability of Paternity Exclusion Based on Restriction Fragment Length Polymorphisms:** Hiroko ITO, Norikazu YASUDA (Div. Genet., Natl. Inst. Radiol. Sci., Chiba) and Hideo MATSUMOTO (Dept. Legal Med., Osaka Med. Coll., Takatsuki)

父親の排除は，母子が特定の男性を父として認知を求めたとき，これを否定する確率である．法医学の領域では，これまで赤血球，白血球，血清，酵素，唾液などの遺伝多型を用いて排除確率を計算している．一方，最近は DNA 制限酵素切断片多型 (RFLPs) の研究が進み，これらに高い排除確率が予想される．またヒトの突然変異率を直接調査する研究では，高い排除確率を示す遺伝標識を選ぶことが重要となろう．今回，第8回ヒト遺伝子地図作製検討会（1985）で報告された RFLPs を基にして排除確率を計算した．これは白人を主とした資料であるが，常染色体上（位置不明も含む）のプローブで排除確率が 40% 以上のものは，INS (62.3%), m33.6 (54.2%), D17S2 (52.4%), DNF15S1 (47.2%), D2S3 (46.8%), D14S4 (42.6%), m33.4 (42.1%) の 7 つであり，このうちすくなくとも 1 プローブ排除しうる確率は 99.2% となった．一方，日本人集団でこれまでよく利用されている遺伝多型で排除確率の高いものは，Gm (40.7%), Gc (38.2%), PGM_1 (26.4%), Rh (23.7%), MLSs (23.5%), ABO (19.2%) であるが，このうちのすくなくとも 1 遺伝標識で排除しうる確率は 87.3% である．HLA については第 9 回国際組織適合性会議の報告から，HLA-B (80.9%), HLA-DR (69.7%), HLA-A (57.5%), HLA-C (42.6%), HLA-DQ (29.5%) という数値が得られた．今後 RFLPs の日本

人の資料が必要なことは言うまでもないが，すでに報告のある種々の遺伝多型についても日本人集団の代表値を定める必要があろう．

Ito, H. *et al.* 1986. *Jpn. J. Human. Genet.* **30**: 261, 1985.

II-29. Genetic Analysis of Dyslipoproteinemias Associated with Ischemic Heart Disease: I. *Sst*I Polymorphism in Apo C-III Gene in Japanese Patients with Myocardial Infarction: **Mieko ONUKI,[1] Yasuko YAMANOUCHI,[1] Yukio IWAMURA,[2] Shigeru TSUCHIYA,[3] Hideomi FUJIWARA,[4] Hiroshi AMAMIYA[4] and Hideo HAMAGUCHI[1]** ([1]Dept. Hum. Genet., [2]Dept. Bact., Inst. Basic Med. Sci., [3]Inst. Comm. Med., Univ. Tsukuba, Ibaraki; [4]Tsuchiura-kyodo Hosp., Tsuchiura)

There seem to be relatively common major genes and polymeric genes for dyslipoproteinemias related to premature coronary artery disease. Besides the mutant gene at the locus for LDL receptor, *Sst*I polymorphism at the 3′ noncoding region of the apo C-III gene in the apo A-I/C-III/A-IV gene cluster has been reported to be strongly associated with hypertriglyceridemia and myocardial infarction in Caucasians. In order to reveal whether *Sst*I polymorphism in the apo C-III gene is also positively associated with myocardial infarction in Japanese, the genotypes for the *Sst*I polymorphism at the 3′ noncoding region of the apo C-III gene were analyzed in 48 Japanese patients with post-myocardial infarction and 49 healthy subjects by Southern hybridization using Apo A-I gene probe gifted from Dr. Humphries. In 48 patients, 21 possessed the genotype *S1S1*, 24 the genotype *S1S2*, and 3 the genotype *S2S2*. Among 49 healthy subjects, 18 possessed the genotype *S1S1*, 22 the genotype *S1S2*, 9 the genotype *S2S2*. Unlike the positive association of the *S2* allele with myocardial infarction in Caucasians (gene frequencies of *S2*: 0.11 in patients *versus* 0.02 in controls), the frequencies of *S2* were high in both patients (0.31) and controls (0.41) without significant difference in the frequencies between two groups. In Japanese, the particular DNA polymorphisms that might be in linkage disequilibrium with putative mutant gene(s) at the apo A-I/C-III/A-IV gene cluster seem to be different from those in Caucasians.

II-30. Genetic Polymorphism of Apo E and Dyslipoproteinemias in Japanese: II. Apo E5 and E7 in Healthy Subjects and Patients with Myocardial Infarction: **Yasuko YAMANOUCHI,[1] Mieko ONUKI,[1] Shigeru TSUCHIYA,[2] Hideomi FUJIWARA,[3] Hiroshi AMAMIYA,[3] Ryunosuke MIYAZAKI[4] and Hideo HAMAGUCHI[1]** ([1]Dept. Hum. Genet., Inst. Basic Med. Sci., [2]Inst. Comm. Med., Univ. Tsukuba, Ibaraki; [3]Tsuchiura-kyodo Hosp., Tsuchiura; [4]Kudanzaka Hosp., Tokyo)

Apo E5 and apo E7 are genetic variants of apo E and have been reported to be present

in about 5% of Japanese patients with hyperlipidemia and ischemic heart disease. The existence of apo E5 and apo E7, however, have not been reported in apparently healthy individuals except for a few family members of the patients with apo E5 or E7. It has been suggested that apo E5 and apo E7 may be closely related to the development of atherosclerosis. The purpose of this study is to investigate the frequency of apo E5 and apo E7 in apparently healthy Japanese adults and patients with myocardial infarction and to analyze serum lipid levels of the individuals with apo E5 or apo E7. Apo E phenotypes were analyzed in 92 Japanese post-myocardial infarction patients and 197 unrelated healthy adults by two-dimensional gel electrophoresis using partially purified VLDL. Apo E5 or E7 was detected in four patients (4.4%) and three healthy adults (1.5%). Among them, four (two patients and two healthy subjects) were heterozygous with apo E3 and the four individuals all are normolipidemic. The other three (two patients and one healthy subject) were heterozygous with apo E2 or E4 and all the three individuals are hyperlipidemic. Since only apo E3 has yet to be associated with any of the lipid disorders among the three common apo E types, the data suggest that apo E5 and apo E7 may not act as a dominant major gene but as a recessive major gene or one of polymeric genes in predisposing one to hyperlipidemia. The data also suggest that the frequency of the individual with apo E5 or apo E7 is of the order of 1% in the Japanese population.

II-31. アカタラセミアマウスの赤血球カタラーゼの分画および等電点電気泳動による分析：佐藤征紀・田中由紀子・緒方正名（岡山大・公衆衛生）. **Properties of Blood Catalase in Acatalasemic Mice Revealed by Chromatofocusing and Isoelectric Electrophoresis:** Yukinori SATO, Yukiko TANAKA and Masana OGATA (Dept. Public Health, Okayama Univ., Okayama)

無カタラーゼ血症の成因としてカタラーゼの生合成障害の問題のほかに，スイス人とマウスでは，赤血球の成熟にともなう活性の消失の促進が観察されており構造の変化が推定されている．ヒトの赤血球カタラーゼは酸化状態により異型性の A・B・C の 3 画分が知られており，マウスにおいてもその存在が想定される．これまでにも正常とアカタラセミアマウスの残余カタラーゼ分子には構造上の差があると考えられてきたが，さらに正常マウスとミュータントマウスでは異型性のカタラーゼ分子が，どのような関係にあるかを調べるために，正常・アカタラセミア同型接合体，ヒポカタラセミア異型接合体マウスの赤血球カタラーゼを DEAE-カラムクロマトグラフで分析したところ，A・B・C の 3 画分に分離された．正常は C が多く，アカタラセミアはやや A 画分が多かった．また，各画分を濃縮して 7°C，pH 3～10 のアガロース等電点電気泳動で分離し，KI 染色を施した結果，各バンドの中心点の平均値で比較すると，A・B は酸性側に近く，C はアルカリ性側に近く認められた．また，A画分で比較すると，アカタラセミアのカタラーゼの等電点は正常マウスのそれよりややアルカリ側に近かった．そして，ヒポカタラセミアはほぼ中間に認められた．この傾向は B・C 画分でも同じであった．以上の実験成績より，アカタラセミアのカタラーゼの蛋白部分に構造上の変異が存在すると考えられる．

II-32. Nonketotic Hyperglycinemia: Kiyoshi HAYASAKA and Keiya TADA (Dept. Pediatr., Tohoku Univ., Sendai)

The glycine cleavage system (GCS) is a major pathway for the catabolism of glycine in vertebrates and is composed of four enzyme components: P-protein, H-protein, T-protein, and L-protein. Hyperglycinemia is associated with decreased catabolism of glycine caused by reduction in the activity of GCS. GCS was investigated in the livers and brains of seven patients with typical nonketotic hyperglycinemia (typical NKH) and in the livers of three patients with atypical nonketotic hyperglycinemia (atypical NKH). The overall activities of GCS were 0.1–0.7 μmol/g prot/hr in the livers (control, 3.8–5.2 μmol/g prot/hr) and 0.1–0.3 μmol/g prot/hr in the brains of the patients with typical NKH (control, 0.5–0.9 μmol/g prot/hr). The overall activities of GCS were 0.7, 1.0 and 1.7 μmol/g prot/hr in the livers of the patients with atypical NKH. Examination of the activities of the individual components in both tissues revealed that the activity of P-protein was undetectable in five and the activity of T-protein was undetectable or extremely low in two patients with typical NKH. In two cases with the atypical NKH, the activity of T-protein decreased and the activities of the components were not analyzed in one case, because of insufficient materials. Our data indicate that NKH is caused by a primary disturbance of any components of GCS and the clinical manifestation may be determined by the degree in the impairment of GCS.

II-33. Cystinuria Diagnosed during Neonatal Period: Takahiro TAHARA and Yoshikatsu ETO (Dept. Pediatr., Tokyo Jikei Univ. Sch. Med., Tokyo)

Cystinuria is an heritable disorder of dibasic amino acids and cystine transport affecting the epitherial cells of the renal tubles and gastrointestinal tract. The heterozygote state reflects true or incomplete recessive inheritance. Present report concerns four cases of cystiuria found in neonatal urinary screening by TLC and discusses genetic variations of excretion and absorption of urinary cystine and dibasic amino acids in renal tubles and intestine in homozygote and heterozygote states. According to Rosenberg's classification, our four cases could be classified into group II, while some of loading test such as lysine loading on these patients did not indicate type II by Rosenberg criteria. Therefore, the classification by Rosenberg could not be applied to Japanese cases, possibly due to race difference. Among six heterozygotes with cystinuria, the urinary excretion of cystine and dibasic amino acids has wide varieties. There are two groups of heterozygote state; one is completely normal and the other shows higher excretion of cystine and lysine with normal excretion of ornithine and arginine, probably indicating incomplete heterozygous state. These clinical and genetic variations in cystinuria could not be explained by simple loading

tests and by Rosenberg classification. Furthermore, the relationship between clinical subtypes and biochemical abnormality should be clarified in future.

II-34. Partial Purification and Characterization of Human Erythrocyte Arginase: **C. HAYAKAWA, M. MAEHARA, N. MIZUTANI and K. WATANABE** (Dept. Pediatr., Nagoya Univ., Nagoya)

Human erythrocyte arginase was partially purified and characterized for the purpose of the enzyme replacement therapy to the patient with argininemia. The purification of arginase from human erythrocyte was carried out according to the method of Vielle Breitburd and Orth. The specific activity of the partially purified enzyme is 22.5 units/mg protein, 1,500-fold greater than that of the crude erythrocyte extract. The molecular weight, estimated by gel filtration on a Sephacryl S-300 column, is 120,000. SDS-polyacrylamide gel electrophoresis shows the presence of one polypeptide band with a molecular weight of 33,000. These results suggest the tetrametric structure of the human erythrocyte arginase. The K_m for L-arginine is 19 mM. L-Lysine and L-ornithine exhibit a competitive type of inhibition with K_i of 12.8 mM and 7.2 mM, respectively. Intact erythrocytes from normal subjects metabolize 4–5 nmol L-arginine/day/10^7 erythrocytes. Erythrocytes from the patient can not metabolize L-arginine at all. Enzyme replacement therapy by erythrocyte exchange is considered to be an effective method for the treatment of the patient with argininemia.

II-35. アルカリホスファターゼ欠損症の臨床所見と遺伝的異質性：多田有希・伊藤文之・衛藤義勝（慈恵医大・小児）. **Clinical Findings and Genetic Heterogeneities of Hypophosphatasia:** Y. TADA, F. ITO and Y. ETO (Dept. Pediatr., Tokyo Jikei Univ. Sch. Med., Tokyo)

アルカリホスファターゼの遺伝的欠損によって発症する hypophosphatasia の自験例 5 例について，臨床的ならびに酵素学的検討を行った．症例は，生下時より頭蓋骨欠損，呼吸不全，痙攣など症状が重篤で新生児期に死亡した同胞例 2 例，生後 1～2 カ月に痙攣，肺炎にて入院時に診断され死に至った 2 例，生後 4 カ月に肺炎にて入院時に診断され，片麻痺，痙攣など残しながらも現在 11 歳に達している若年型と思われる例の計 5 例である．新生児期に死亡する症例は X 線像所見を含め，症状はきわめて重篤であり，従来いわれている乳児型とは区別して新生児型あるいは重症乳児型として取り扱われるべきであろう．新生児型の患児の剖検時摘出臓器および胎盤におけるアルカリホスファターゼ活性は，肝臓，腎臓，胃においてのみ低下しており，小腸，胎盤では保たれていた．これは従来の報告と一致している．また，アルカリホスファターゼ活性の低下している上記組織では，リン酸エステルの蓄積がコントロールに比して有意に増加していた．ホスホエタノールアミン含量も，肝臓，腎臓で有意な差が認められた．新生児型，乳児型，若年型では，臨床症状の出現状況，X線像所

見に大きな差があるにもかかわらず，血清アルカリホスファターゼ活性値に差の認められないことは興味あるところで，今後，組織レベルにおける酵素学的検討を進め，その遺伝的差異を含め病態の解明を行っていきたいと考えている．

II-36. Analysis of Oligosaccharides Excreted in the Urine of the Mother of a Family Affected by Sialidosis: Excretion of Neutral Galactosyl Oligosaccharides: **Yukitoshi TAKAHASHI, Yutaka NAKAMURA, Tadao ORII** (Dept. Pediatr., Gifu Univ., Gifu), **Takashi OSHIRO, Kiyotake HIRAYAMA** (Dept. Pediatr., Ryukyu Univ., Okinawa), **Makoto NAITOU** (Dept. Ophthalmol. Ryukyu Univ., Okinawa) **and Tooru KUDOH** (Dept. Pediatr., Sapporo Med. Coll., Sapporo)

Last year, we reported a family affected by sialidosis (normosomatic type). As father and mother had cherry red spots, we examined urinary oligosaccharides from members of the family. The amount of excreted oligosaccharides in the urine of the mother was 17.1 nmol/mg creat. and neutral galactosyl-oligosaccharides detected in G_{M1} gangliosidosis urine were also found in the urine of the mother. Activities of lysosomal enzymes except for β-galactosidase were normal in fibroblast homogenates of the mother, though neuraminidase activities using 4MU and sialyl lactose as substrates were about half of controls. As regards β-galactosidase, 4MU-β-galactosidase, G_{M1} ganglioside-β-galactosidase and galactosyl ceramide-β-galactosidase activities were within normal limits, but galactosyl oligosaccharides-β-galactosidase was 4.1 (control : 12.7 ± 0.8, G_{M1} gangliosidosis type 3 : 5.1) nmol/mg prot./20 hr. These results suggested that β-galactosidase of the mother has mutated enzyme activity against galactosyl-oligosaccharides.

II-37. Analysis of Genes Coding for 21-Hydroxylase in Patients with Congenital Adrenal Hyperplasia Due to the Deficiency of This Enzyme: **Jun NAKURA,[1] Tetsuro MIKI,[1] Ken-ichi FUKUCHI,[1] Kazuo SHIMIZU,[2] Osamu NOSE,[2] Isamu NISHISHO,[3] Hideo TATEISHI,[3] Shin-ichiro TAKAI,[3] Tasuku HONJO[4] and Yuichi KUMAHARA[1]** ([1]Dept. Med. Geriat., [2]Dept. Pediatr., [3]2nd Dept. Surg., Osaka Univ., Osaka; [4]Dept. Med. Chem., Kyoto Univ., Kyoto)

Recently the cDNA of 21-hydroxylase (OHase) has been cloned, and it is confirmed that two 21-OHase genes (gene A and B) are located in the MHC class III region. To study the abnormality of these genes in patients with congenital adrenal hyperplasia due to 21-OHase deficiency, we carried out Southern blot analysis using human cDNA probe of this enzyme (obtained from Dr. White) on DNA samples from five unrelated patients with salt-losing type of this syndrome. After digestion with *Taq*I, all samples from normal individuals yielded 2 hybridizing bands (4.0 and 3.4 kb). According to Dr. White's paper,

the gene B is functionally active while gene A is inactive, and 4.0 kb band is derived from the gene B. Complete absence of the 4.0 kb band was found in 2 out of the 5 patients, suggesting that loss of DNA portion containing 21-OHase gene B was the cause of 21-OHase deficiency in them. Parents of one of these 2 patients with deleted 21-OHase B gene were consanguinous. Analysis of this family revealed that the deletion of B gene was linked to HLA types Aw24, Bw61 and Cw3. In the remaining 3 patients, small variations in B gene seemed responsible for 21-OHase deficiency.

II-38. Molecular Genetic Study of 21-Hydroxylase Deficiency: Fumiki HARADA, Tomohisa IWANAGA, Takehiko SASAZUKI (Dept. Genet., Med. Inst. Bioregul., Kyushu Univ., Fukuoka) **and Kikuo TSUKAMOTO** (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo)

Two genes, 21-hydroxylase A (21-OHA) and 21-hydroxylase B (21-OHB) were localized to the class III region of HLA close to C4A and C4B. Eight DNA clones were isolated from a human genomic library using a C4B cDNA probe. Three out of the 8 clones hybridized with a 21-OHase cDNA probe. Restriction mapping and hybridization analysis demonstrated that the two genes, 21-OHA and 21-OHB, carried the *Taq*I fragments of 3.2 and 3.7 kb respectively. Southern blot analysis of 12 patients with 21-OHase deficiency revealed that 2 out of 6 salt loosers were missing the 3.7 kb *Taq*I fragment and that in DNA from 2 salt loosers and a virilizer, the 3.7 kb fragment was always fainter than the 3.2 kb fragment, whereas the other patients and normal controls carried both of these fragments in the same intensity. This indicated that the 3.7 kb *Taq*I fragment was absent in about 30% of the affected haplotypes, and a family study confirmed the Mendelian segregation for the absence of 3.7 kb fragment. Similarly, it was reported that DNA from Caucacian patients, homozygous for HLA-A3-B47-DR7, did not carry the 3.7 kb *Taq*I fragment, which was explained as a deletion of 21-OHB gene. However, Southern blot analysis using *Bgl*II clearly demonstrated the 11 kb fragment corresponding to 21-OHB gene, which suggested that the absence of 3.7 kb *Taq*I fragment was not necessarily due to the large deletion of the 21-OHB gene, but due to the other mutation of the gene.

II-39. 血液による Lesch-Nyhan 症候群保因者の判定：鎌谷直之・山中　寿・西岡久寿樹（東京女医大・リウマチ痛風センター）. **Detection of Carriers of Lesch-Nyhan Syndrome Using Blood:** N. KAMATANI, S. YAMANAKA and K. NISHIOKA (Inst. Rheum., Tokyo Women's Med. Coll., Tokyo)

[目的]　今まで fibroblast や毛根細胞で行われていた，Lesch-Nyhan 症候群の保因者の判定を，血液でできるようにする．[方法] ヒトの切除したへんとうを培養して interleukin 2 を含む液を作製

する．被験者より末梢血をヘパリン採取し，これより単核細胞を取り出した後培養する．最初は PHA で刺激し，後には interleukin 2 を含む培養液中で培養すると，T 細胞が 2 週間以上ふえつづける．適当な時期に 6 日ほどかけて T 細胞の 6-thioguanine に対する抵抗性を調べる．一般検査には 5μM の 6-thioguanine 溶液中で培養する．[結果]　正常 T 細胞は 6-thioguanine により死滅し Lesch-Nyhan の T 細胞は死なない．その差は 1,000 倍以上である．保因者はモザイクであるから，抵抗性の細胞が生き残り，その後，それらが活発に増殖をはじめる．正常と保菌者の判定は容易で，ヘパリン採血後 4 日ほど経過したサンプルでも判定可能．判定まで約 2 週間を要する．[考察]　毛根細胞を用いる方法より確実さの点で，fibroblasts を用いる方法より被験者への負担，判定の早さの点ですぐれている．Lesch-Nyhan 症候群保因者の判定は遺伝相談上重要な検査であり，この新しい方法の価値は高いと思う．

II-40.　日本人型 APRT 欠損症の疾患遺伝子の起源：鎌谷直之・登　勉・山中　寿・西岡久寿樹（東京女医大・リウマチ痛風センター），**藤森　新・赤岡家雄**（帝京大・医・2 内）．**Origin of the Mutant Gene for the Japanese Type of APRT Deficiency:** N. KAMATANI, T. NOBORI, S. YAMANAKA, K. NISHIOKA (Inst. Rheum., Tokyo Women's Med. Coll., Tokyo), S. FUJIMORI and I. AKAOKA (2nd Dept. Intern. Med., Teikyo Univ. Sch. Med., Tokyo)

[目的]　日本人型 APRT 欠損症（部分欠損症に 2,8-dihydroxyadenine 結石症を伴う）の診断法を確立し，なぜ部分欠損症が結石症を伴うかを解明する．また，この遺伝疾患がなぜ欧米で見られないかを考察し，疾患遺伝子の歴史と人種の歴史との関係を考察する．[方法と結果]　13 家系の 2,8-dihydroxyadenine 家系（すべて日本人）よりサンプルを得た．このうち，4 家系では APRT 完全欠損症，9 家系では APRT 部分欠損症に結石を伴っていた．前者の家族 5 名は，やはり APRT 部分欠損で保因者と考えられた．9 家系の日本人型 APRT 欠損の患者 T 細胞は，adenine analogs に 100 倍以上抵抗性であり，正常人および完全欠損の保因者と明らかに区別でき，診断法として価値が高いと思われた．EBV による B cell line のレベルでも，日本人型の細胞は完全欠損の細胞と同じく，しかし，正常細胞や完全欠損の保因者の細胞と異なり，adenine analogs に抵抗性であった．6 家系の日本人型欠損より APRT を部分精製した結果，変異 APRT は PRPP に対する親和性の著しい低下，熱安定性亢進をはじめ，いくつかの点で正常酵素とは異なった，しかも驚くべきことに，6 つの変異酵素はすべて同じ変異性格を持っていた．[結論]　日本人型 APRT 欠損症の疾患遺伝子は共通であろうと推定される．

II-41.　Swelling of Lymphocytes under Thymidylate Stress: Ichiro MURANO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Blood samples from six volunteers, two males and four females, were cultured for 96 hr under the following six conditions. 1) MEM: Eagle's minium essential medium. 2) MEM+BrdU: 5-Bromodeoxyuridine was added to MEM at a final concentration of 20 mg/ml for the last 6 hr. 3) MEM+aphidicolin: 0.2 μM aphidicolin, dissolved in 0.2%

ethanol, was added to MEM for the last 26 hr. 4) MEM+FUdR: 0.1 μM fluorodeoxyuridine was added to MEM for the last 24 hr. 4) MEM+MTX: 10 μg/ml methotrexate was added to MEM for the last 24 hr. 6) MEM-FA: MEM without folic acid (Nissui). Chromosomes slides were prepared in a usual manner. The diameter of 200 lymphocytes in each condition was measured under a microscope equipped with an ocular micrometer. The mean+SD values for the diameter of 1,200 lymphocytes from 6 individuals under each condition was: MEM, 13.7±3.07 μm; MEM+BrdU, 14.3±3.36 μm; MEM+aphidicolin, 16.1±4.43 μm; MEM+FUdR, 20.5±5.93 μm; MEM+MTX, 20.6±6.08 μm; MEM-FA, 15.5±4.66 μm. These results indicate that the swelling of lymphocytes occurs under thymidylate stress (4, 5, 6) and the presence of aphidicolin. Distribution of cellular DNA content was studied by cytometry in MEM and MEM+FUdR. High $G_{0/1}$ and low G_2+M peaks were detected in each condition. There was one more peak close to the $G_{0/1}$ peak in MEM+FUdR.

II-42. 均衡転座型染色体異常保因者に対する胎児診断例の検討：香山文美・千谷東海・野末 順・佐藤孝道・水野正彦（東大・医・産婦人）. **Prenatal Diagnosis of Fetuses of Translocation Carriers:** F. KAYAMA, T. CHITANI, J. NOZUE, K. SATO and M. MIZUNO (Dept. Obstet. Gynec., Univ. Tokyo, Tokyo)

1973 年 1 月から 1985 年 6 月までの 12 年半に当科で施行した，羊水細胞を用いた妊娠 24 週未満の胎児診断 608 例のうち，均衡転座型染色体異常を適応とした 24 例 (3.9%) について検討した．診断の結果は，患児 6 例 (25.0%)，保因児 8 例 (33.3%)，正常児 10 例 (41.7%) であり，すべて出生後あるいは中絶胎児については正診であったことが確認されている．24 例のうち母親が保因者であるものは 16 例 (66.7%)，父親が保因者であるもの 7 例 (29.2%)，両親ともに保因者であるもの 1 例 (4.2%) であった．母親が保因者の場合は，患児 4 例 (25.0%)，保因児 6 例 (37.5%)，正常児 6 例 (37.5%) であり，父親の場合は，患児 2 例 (28.6%)，保因児 1 例 (14.3%)，正常児 4 例 (57.1%) であった．転座の型別にみると，ロバートソン転座 12 例では，患児 2 例 (16.6%)，保因児 5 例 (41.7%)，正常児 5 例 (41.7%) であり，相互転座 12 例では，患児 4 例 (33.3%)，保因児 3 例 (25.0%)，正常児 5 例 (41.7%) であった．また，ロバートソン転座のうち，10 例は母親が保因者で，父親が保因者であるものは 1 例のみであった．一方，相互転座では母親，父親が保因者であるものはそれぞれ 6 例ずつと同数であった．以上より，相互転座の場合，患児，保因児，正常児の割合がほぼ同数になるのに対し，ロバートソン転座では，患児の割合が少なくなることが推測された．

II-43. 経腟的絨毛採取による胎児診断の臨床応用：佐藤孝道・千谷東海・野末 順・香山文美・水野正彦（東大・医・産婦人）. **Prenatal Diagnosis Using Chorionic Villus:** Kodo SATO, Tokai CHITANI, Jun NOZUE, Fumiyoshi KAYAMA and Masahiko MIZUNO (Dept. Obstet. Gynec., Univ. Tokyo, Tokyo)

経膣的絨毛採取（以下，CVS）は羊水穿刺に代わる方法として注目を受けているが，なお検討を要する点も多い．われわれは 2 年間にわたる基礎的検討を踏まえ，わが国で最初に CVS の臨床応用を開始した．基礎研究からは，1) 妊娠 8～10 週が絨毛採取に最適であること，2) 採取時超音波電子スキャンによるガイドの重要性，3) われわれの開発した絨毛採取用 S-1 カテーテルの有用性，4) 約 2 時間の培養で染色体標本作成が可能なこと，5) この標本作成法によると母体組織の混入による影響が少ないことが明らかとなった．臨床的には，妊娠 7 週，9 週，9 週の 3 例に CVS を実施し，それぞれとくに副作用もなく染色体分析が可能であった．うち 1 例は，46,XY/47,XXY のモザイクの可能性があると診断されたために，念のため羊水穿刺を行ったが結果は 46,XY であった．前記の 3 例以外に 2 例 CVS を試みた症例があったが脱落膜組織しか採取できず，CVS を中止した．これらの症例は妊娠 12 週と 9 週であったが，CVS の試みの後の妊娠経過は順調である．以上から，CVS の臨床応用の意義および可能性はきわめて大きいと考えられるが，さしあたりは適応を 1) 転座型染色体異常者，2) 42 歳以上の高齢妊娠，3) 絨毛により胎児診断可能な先天性代謝異常症保因者など再発危険率が高い症例に限り，慎重に実施する必要があろう．

II-44. 出生前診断を施行した 3 症例—水腎症，多嚢胞腎と非血友病 B_M—：松井　晶・栗林俊夫（伊勢崎市民病院・小児），**名古純一**（伊勢崎市民病院・産婦人），**吉岡章・大久保芳明**（奈良医大・小児），**是澤光彦**（筑波大・臨床医・産婦人），**神谷忠**（名大・医・一内），**松田健史**（富山医薬大・医・一解剖）．**Three Cases of Prenatal Diagnosis: Hydronephrosis, Polycystic Kidney and Non-Hemophilia B_M:** A. MATSUI, T. KURIBAYASHI (Dept. Pediatr., Isesaki City Hosp., Gunma), J. NAKO (Dept. Obstet. Gynec. Isesaki City Hosp., Gunma), A. YOSHIOKA, Y. OOKUBO (Dept. Pediatr. Nara Med. Univ., Nara), M. KORESAWA (Dept. Obstet. Gynec., Tsukuba Univ., Ibaraki), T. KAMIYA (Dept. Intern. Med. Nagoya Univ., Aichi) and T. Matsuda (Dept. Anat., Toyama Med. Pharmaceut. Univ., Toyama)

出生前に，超音波検査により発見された水腎症，多嚢胞腎の各 1 症例と，胎児採血により非血友病 B_M と診断された 1 症例の計 3 症例を経験したので報告する．1) 症例 1：妊娠 22 週の超音波検査で，胎児の左腎盂に軽度の拡張がみられた．その後，妊娠週数が進むとともに，左腎盂の拡張は増強し，出生後 IVP により左水腎症が確認された．生後 11 カ月になるが，発育は順調である．2) 症例 2：第 1 子（兄）が，両側の多発性嚢胞腎，肺拡張不全にて死亡．第 2 子妊娠の 39 週の超音波検査で，胎児の右腎に多発性の嚢胞が認められた．左腎は代償性に肥大していたが，嚢胞はみられなかった．生後 1 カ月になるが，発育は順調である．3) 症例 3：第 1 子（兄）が血友病 B_M で，第 2 子妊娠中に遺伝相談を行い，家族の強い要望により出生前診断を行った．妊娠 16 週の羊水検査では 46,XY の男性胎児と診断．妊娠 21 週，胎児鏡により臍帯穿刺採血を行うも採取不能のため，B-Scope 下に胎児肝内血管穿刺を行い，約 1.5 ml の血液を得た．IX: C 3.6 u/dl IX:Ag 4.8 u/dl，ウシ脳 PT 71″（正常 31.5″，第 1 子 110″）であり，非血友病 B_M と考えられた．昭和 59 年 9 月 10 日，出生，臍帯血 IX:C 55 u/dl, IX:Ag 30 u/dl，生後 10 時間目の静脈血 IX:C 30 u/dl, IX:Ag 25 u/dl，ウシ脳 PT 42.5″ (45″)，生後 6 カ月目の IX:C 66 u/dl, IX:Ag 65 u/dl，ウシ脳 PT 59″ (53″) で，非血友病 B_M 児であることが確認された．

II-45. Regional Assignment of the Gene Encoding Lymphocyte Cytosol Polypeptide with Molecular Weight of 64,000 (LCP1) to 13q14.1-14.3: **I. KONDO,[1] T. IKEUCHI,[2] I. NISHIGAKI,[3] H. TAKITA,[1] K. FUJIKI,[4] A. NAKAJIMA,[4] Y. TAKAHASHI,[5] K. YAMAMOTO[2] and H. HAMAGUCHI[1]** ([1]Univ. Tsukuba, Ibaraki; [2]Tokyo Med. Dent. Univ., Tokyo; [3]Kyoto Pref. Med. Sch., Kyoto; [5]Juntendo Univ., Tokyo; [5]Toyohashihigashi Natl. Sanatorium, Aichi)

Lmphocyte cytosol polypeptide with molecular weight of 64,000 (LCP1) is a polymorphic protein detected in lymphocytes by two-dimensional gel electrophoresis and the phenotype is determined by two common alleles at an autosomal locus. In order to assign the gene for LCP1 (*LCP1*) to a particular chromosomal region, we have studied the phenotype and protein amount of LCP1 in patients with various kinds of chromosome anomalies and their parents. A mode of inheritance of the phenotype of LCP1 in a patient with a deletion of chromosome 13 was unusual, suggesting that one gene for LCP1 is deleted in the patient. Since a gene for esterase D(*ESD*) has been assigned to chromosome 13q14.1 and since the locus for *ESD* is linked to the loci for retinoblastoma and Wilson diesease, we have studied on linkage between *LCP1* and *ESD*. No recombinations were observed in four informative families, giving a summed lod score of 4.221 at recombination fraction 0. To determine more precise localization of *LCP1* using deletion mapping, we have studied phenotypes and protein amount of LCP1 in patients with chromosome 13 deletion carring different break points and their parents. (1) A patient with retinoblastoma and a deletion of 13q, del(13)(q12.3–21.2) had a type 1 of LCP1 and the protein amount was a half of the mean amount of her parents. One gene for esterase D was also deleted in the patient. (2) A patient with a deletion 13q, del(13)(q14.3–31) but without retinoblastoma had a type 1 of LCP1 and the protein amount in the patient was similar to that of his mother. The sedata indicate that the gene for LCP1 is assigned to chromosomal region 13q14.1–14.3 and may be a useful genetic marker for preclinical diagnosis of retinoblastoma and Wilson disease.

II-46. Review of the Methodology Used in Gene Mapping on Human UDPGal 4-Epimerase: **Jun OIZUMI** (Natl. Child. Med. Res. Cent., Tokyo) **and Min LIN** (Div. Med. Genet., Univ. South. Calif., L.A.)

The gene locus for human UDPGal 4-epimerase has been assigned to chromosome 1 by our group. The use of human-rodent somatic cell hybridization has been a powerful method for human gene mapping. Hybrid cell clones between human diploid cells and mouse cells deficient in either hypoxanthine phosphoribosyltransferase or thymidine kinase were analyzed for the expression of human UDPGal 4-epimerase. The gene for

UDPGal 4-epimerase was also observed to be syntenic with enolase-1. Furthermore, the use of human reciprocal translocation cell strains for regional gene mapping was discussed.

II-47. Localization of a Human Prealbumin Gene by *In Situ* Hybridization: Yoshihiro JINNO, Tsutomu KAMEI, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki), **Shukuro ARAKI** (1st Dept. Intern. Med., Kumamoto Univ., Kumamoto) **and Kazunori SHIMADA** (Dept. Biochem., Kumamoto Univ., Kumamoto)

A cDNA coding for human prealbumin (PA), a single amino acid substitution of which is associated with hereditary amyloidosis, has been cloned by Mita *et al.* (1984). Using this cDNA as a probe we have intended to determine the localization of the PA gene on a chromosome by *in situ* hybridization. A histogram depicting the distribution of silver grains from the 137 cells examined revealed the highest score on the short arm of chromosome 1, 1p31, but the grains at this region was only up to 7.8% of total grains due to a rather high background. The DNA prepared from a neuroblastoma cell line with a deletion of the segment distal to 1p3 did not show any gene dose effects. Meanwhile, Wallace *et al.* (1985) localized the human PA gene to chromosome 18 by DNA analysis of mouse/human somatic cell hybrids. Then, we examined the more precise localization of the gene by Southern blot analysis of DNAs derived from cells or cell lines with various aberrations of chromosome 18, which had a karyotype of 46,XY, rec(18), dup, 46,XX,psu dic(18), 46,XX, inv(18)(p11.2q21.3), 47,XX,+18, or 46,XX,18p−. The DNA analysis on them did not again show any gene dose effects. This may indicate that the locus of the PA gene is not on the chromosome 18. The DNAs prepared from chromosome 1 isolated by a cell sorter or cells with various aberrations of chromosome 1 should be further analyzed.

II-48. The Analysis of Nine Genes on Human Chromosome 19 Using Monochromosomal Hybrid: Yasufumi KANEDA, Tsuyoshi UCHIDA, Yoshio OKADA (Inst. Mol. Cell. Biol., Osaka Univ., Osaka) **and Michihiro YOSHIDA** (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

We have mapped human elongation factor 2 (EF-2) gene to chromosome 19. Then the monochromosomal hybrid, HM76Dd, was isolated which retained human chromosome 19 as the only human chromosome. On subcloning HM76Dd, subclone 40-6 was isolated which has lost the sensitives to poliovirus, echo 11 virus and RD114 virus. However, subclone 40-6 has retained the other genes located on human chromosome 19, which are EF-2, LDLR, C3, GPI, PEPD, and MANB. When the chromosomes of subclone 40-6 were stained with Hoechst 33258, the C-band of the long arm of human chromosome 19 was

found to be amplified. This was also confirmed by C-staining method using $Ba(OH)_2$. Giemsa-11 staining revealed that in the subclone 40-6 a mouse chromosomal fragment was translocated to the long arm of human chromosome 19. *In situ* hybridization using human *Alu* or mouse B1 repeated sequences as probes showed that a translocation between a mouse chromosome and a part of the long arm of human chromosome 19 had occurred. It is supposed that the region from 19q13.1 to q*ter* has been lost and that a mouse chromosomal fragment has replaced the lost region. From these results we conclude that the three virus sensitivities (PVS, ELLS and RDRC) are mapped to the region from 19q13.1 to q*ter*, while the other six genes (EF-2, C3, LDLR, GPI, PEPD and MANB) are located in the region from 19p*ter* to q12. Apart from the mapping study, these monochromosomal hybrids are very useful for constructing gene library of human chromosome 19.

II-49. ヒト筋型アルドラーゼ遺伝子の染色体へのマッピング：吉田迪弘[1]・久木田明子[2]・榊原 稔[3]・向井常博[3]・堀 勝治[3] ([1]北大・理・染色体研，[2]佐賀医大・共同研，[3]佐賀医大・生化). **Chromosome Assignment of the Human Structural Gene for Aldolase A:** Michihiro C. YOSHIDA,[1] Akiko KUKITA,[2] Minoru SAKAKIBARA,[2] Tsunehiro MUKAI[2] and Katsuji HORI[2] ([1]Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo; [2]Dept. Biochem., Saga Med. Sch., Saga)

ヒト筋型（A型）アルドラーゼ遺伝子（ALDOA）の染色体上へのマッピングを，ヒト×マウス細胞雑種クローンパネルを用いて，ノーザン・サザンブロット法および *in situ* ハイブリッド法により行った．ALDOA プローブは A 型サブユニットに対し作られた cDNA クローンより分離したもので，ヒト・マウス間で相同性の低い 3′-noncoding 領域の小断片 DNA（220 塩基対）を主として用い，ヒトアルドラーゼのみを特異的に検出した．ALDOA 遺伝子には多数の偽遺伝子が知られているのでノーザンブロット法によりこれらを区別し，サザンブロット法により ALDOA の存在を確かめた．その結果，ALDOA は no. 16 染色体上にマッピングされ，さらに，*in situ* ハイブリッド法によりその局在部位を 16q22→q24 ときめた．

II-50. Linkage Analysis between the Loci for the Sixth and Seventh Components of Human Complement: Katsushi TOKUNAGA, Georg DEWALD, Keiichi OMOTO (Dept. Anthropol., Tokyo Univ., Tokyo) **and Takeo JUJI** (Blood Transfus. Service, Tokyo Univ. Hosp., Tokyo)

A Japanese family material comprised of 146 matings with 425 offsprings was typed for complement components C6 and C7 using polyacrylamide gel isoelectric focusing and immunoblotting. Three common C6 alleles *C6*A*, *C6*B*, and *C6*B2* and four rare alleles *C6*A3*, *C6*M1*, *C6*M11*, and *C6*B3* were observed. Inheritance of *C6*M11* and *C6*B3* was first confirmed. Three common C7 alleles *C7*1*, *C7*2*, and *C7*4* (identified by P.J.

Lachmann) were observed. The allele frequencies for *C7*1*, *C7*2*, and *C7*4* in 276 unrelated parents were 0.875, 0.087, and 0.038, respectively. Forty-three informative children were obtained and no recombinants were observed. The maximum lod score was 8.47 at recombination fraction $\theta=0.00$. Lod scores were more than 6.5 at recombination fractions less than 0.10. Consequently the close linkage between C6 and C7 loci was confirmed. A total of 552 C6-C7 haplotypes in the parents were used for an association analysis. No significant linkage disequilibrium between C6 and C7 loci was found except a weak association of *C6*B* with *C7*4* ($\chi^2=3.55$, $0.05<p<0.10$). A pedigree indicating the existence of a quantitatively deficient allele of C7 (*C7*Q0*) is also presented.

II-51. Gene Dosage Study of Coagulation Factors XII (F12) and XIII Subunit A (F13A) in a Case with Partial Monosomy 6p: **K. NARAHARA, Y. TAKAHASHI, Y. WAKITA, K. KIKKAWA, S. KIMURA, H. KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama), **R. KASAI** (Asahigawa Jidoin Hosp. Handic. Child., Okayama), **Y. SENO and Y. NISHIBAYASHI** (Dept. Pediatr., Matsuyama Red Cross Hosp., Matsuyama)

F12 has been assigned to 6p23→pter. Linkage studies in males have recently indicated that F13A is also located on 6p, 17 or 18 cM distally to the HLA locus. We studied gene dosage effects for F12 and F13A in a 5-month-old infant with duplication-deficiency resulting from a maternal pericentric inversion of chromosome 6, whose clinical features were similar to those of the partial trisomy 6q syndrome. The karyotype was 46,XX, rec(6),dup q, del p, inv(6)(p2308q25.1)mat. GLO was type 1, and HLA typing showed no loss of the loci (A2, A26, BW35, B−, CW3, CW7, DR1 and DR4). The activity of F12 was low (46%). In view of the parent's values (42% for the mother and 56% for the father), however, the case was consistent with the normal gene dosage. The phenotype of F13A was type 1, and the activity of F13 was normal (80%). These results suggest that both F12 and F13A can be excluded from 6p2308→pter. In conjunction with the linkage data, it seems likely that the two loci exist on the proximal half of the 6p23 band as a gene cluster.

II-52. Linkage Relationship of the Gene for Coagulation Factor XIII-A with HLA-region Genes: **Toshinori NISHIGAKI, Katsushi TOKUNAGA, Keiichi OMOTO** (Dept. Anthropol., Univ. Tokyo, Tokyo), **Takeo JUJI** (Blood Transfus. Service, Tokyo Univ. Hosp., Tokyo) **and Norikazu YASUDA** (Div. Genet., Natl. Inst. Radiol. Sci., Chiba)

The linkage analysis between the locus for coagulation factor XIII-A (F13A) and HLA

region genes (HLA-A, -C, -B) was performed in Japanese. In females, lod scores were negative at any recombination fraction (θ). Significantly negative lod score (< -2.0) at $\theta=0.10$ excluded the linkage closer than 10 centimorgan to HLA. In males, no significantly positive lod score (>3.0) was found at all values of θ ($Z_{max}=0.33$ at $\theta=0.30$), and at the recombination fraction of 0.05 the lod score was significantly negative (-4.43), excluding the linkage closer than 5 centimorgan to HLA. The chi-square value for a test of heterogeneity for θ was 0.65 with d.f.$=38$, which was not significant. There have been conflicting reports concerning the linkage between the locus for F13A and HLA. Board *et al.* (1984) and Olaisen *et al.* (1984, 1985) proposed a linkage between F13A and HLA loci in males. In the present study, however, the results provided no evidence for close linkage between F13A and HLA genes. There have been a few reports also presenting nagative evidences for the linkage. The reason for these different conclusions is not clear. Further investigations may be necessary in order to conclude that F13A is linked to HLA on the short arm of chromosome 6.

II-53. X-Linked Dominant Control of F-Cells in Childhood and Adult Life: **Kazuo MIYOSHI** (Okinaka Mem. Inst. Med. Res., Tokyo), **Yoshikado KENETO, Hisaomi KAWAI, Hiraoki OHCHI, Shinji NIKI, Kiyoshi HASEGAWA, Katsuhito ADACHI, Akira SHIRAKAMI** (1st Dept. Intern. Med., Tokushima Univ., Tokushima) **and Toshinao YAMANO** (2nd Dept. Intern. Med., Kochi Med. Sch., Kochi)

We reported previously (1977) "HPFH Tokushima type" in Japanese adult over 15 years of age, which is similar to the so-called Swiss type, but is characterized by a high incidence, roughly 10% of the population for males and 20% for females, and apparent X-linked dominant inheritance. In this paper, we elucidated that the similar HPFHs reported in various countries including Swiss can be regarded as the same category to our HPFH in their incidence and inheritance. The levels of HbF of general healthy adult are distributed equally between 0.10–3.60% in HbF and 0.2–30% in F-cells in all related papers. The low incidence (1–3%) of individuals with high HbF described in the reported papers can be estimated as high as the incidence of ours, also two times higher in females than males, by setting the upper limit of normal range at a low level as 0.70% for HbF or 4.3% for F-cells. Many female individuals with high HbF were seen in the families described in some of the papers and the mode of inheritance was not incompatible with X-linked dominant one. We also reported (1979) the childhood high F-cell which is observed in individuals of 15 years of age and below with a percentage of 10% for males and 20% for females, in addition to the HPFH. This childhood high F-cell is probably determined genetically, also linking to X chromosome. Two regulator genes on human X chromo-

some, which regulate the developmental switching of HbF to HbA in the period of young adult and of childhood, respectively, are newly speculated, in addition to the controller genes assumed to be present intergenically in the region of β-gene cluster on number 11 chromosome.

II-54. Characterization of a 3.5 kb Y-Specific Repeated Fragment: Yutaka NAKAHORI, Kônosuke MITANI and Yasuo NAKAGOME (Dept. Congen. Abnorm. Res., Natl. Child. Med. Res. Cent., Tokyo)

A cloned 3.5 kb Y-specific repeated fragment was completely sequenced. The total length was 3,564 bp. The whole sequence was proved to consist of multiples and variants of a 5 bp basic unit (TTCCA). This fragment contains an unusual combination of restriction sites, that is, it has 64 *Taq*I sites and 74 *Hinf*I sites and very few for another enzymes now currently used. In those *Taq*I and *Hinf*I sites, 45 were revealed to be overlapped. These finding will confirm our previous presentation about "unequal crossover" and "base substitution" events on this repeated fragment.

II-55. Structure and Organization of Human Immunoglobulin Variable Region of the Heavy Chain: Tatsuo KINASHI, Fumihiko MATSUDA, Kwang Ho LEE, Mieko KODAIRA,* Takafumi NOMA and Tasuku HONJO (Dept. Med. Chem., Kyoto Univ., Kyoto; *Radiat. Effects Res. Found., Hiroshima)

Diversity of variable region is generated by the following mechanisms: 1) the number of the germ-line V segments, 2) the recombinational joining of V-(D)-J segments, and 3) somatic mutation. Since the structure and the organization of human heavy chain variable segments (V_H segments) are unknown, we analyzed human V_H segments. Southern blot analysis in human placental DNA using probes represented V_H subgroup 1, 11, and 111 revealed that about 20, 10, and 30 DNA fragments belonged to subgroup 1, 11, and 111, respectively. A partial library of human placental DNA cloned in the cosmid vector was screened with the above-mentioned probes. About 1,100 kb of V_H regions were cloned and 62 V_H segments were identified. It was found that most of V_H segments detected by Southern blot analysis were cloned. The detail analysis by the restriction enzyme map and DNA sequence showed the following points: 1) Most of V_H segments of different families intermingled in the human V_H locus. 2) The transcriptional orientations of 6 V_H segments on the same clone were identical. 3) 4 out of 6 V_H segments were pseudogenes 4) A unit containing 2 V_H segments was tandemly repeated on the same clone.

II-56. Cloning and Structural Analysis of Human OTC Gene: Akira HATA,[1] Teruhisa TSUZUKI,[1] Kazunori SHIMADA,[1] Masaki TAKIGUCHI,[2] Masataka MORI[2] and Ichiro MATSUDA[3] ([1]Dept. Biochem., [2]Inst. Med. Genet., [3]Dept. Pediatr., Kumamoto Univ., Kumamoto)

Deficiency of ornithine transcarbamylase (OTC) is an X-linked severe inborn error of metabolism. To analyze molecular basis of the OTC deficiency, we initiated cloning of normal human OTC gene. A rat OTC cDNA, which shares 88% DNA homology with the sequence of the human OTC cDNA in the coding region, was used as a probe. 1) Six phage clones were isolated from a human genomic library and were revealed to cover two different parts of the genomic DNA. By chromosome walking, one more phage clone covering a DNA region present between these two genomic DNAs was isolated. A series of seven clones were demonstrated to cover a 65 kb-length contiguous genomic DNA. 2) Absence of the 5′- and 3′-end regions of the OTC gene within the 65 kb-length DNA was demonstrated by hybridization with the synthetic oligomers having the 5′- or 3′-nucleotide sequences of the human OTC cDNA. Accordingly, by chromosome walking, we further isolated phage clones covering 20 kb upstream and that covering 15 kb downstream, respectively, of the 65 kb-length DNA. All together, we isolated an about 100 kb-length contiguous genomic DNA of the OTC gene. Our 5′-end clone was hybridized with the 5′-end synthetic probe. 3) Sequence analysis of the 5′-end region revealed presence of the putative TATA and CAT boxes.

II-57. Gene Analysis of HPRT Deficiencies: Shougo ISHII, Kousaku OHNO, Isematsu EDA and Kenzou TAKESHITA (Div. Child Neurol., Tottori Univ., Yonago)

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) genes of a case of Lesch-Nyhan syndrome (LNS) and a family of hereditary gout were analyzed by Southern blotting technique. The case of LNS indicated a deletion of exon 2, and one patient of the hereditary gout, his parents and siblings showed the same profiles as controls in Southern blotting patterns with restriction enzymes: *Pst*I, *Hinc*II, *Xho*I and *Alu*I. Yang *et al.* reported 5 cases of LNS with several gene mutations (deletion *etc*), but no specific region for LNS was identified in exons. Our results of a deletion of exon 2 on LNS showed different abnormalities from the report of Yang *et al.*, and also could not indicate any specific mutation. Although several hypotheses were proposed to explain the neurological symptoms of LNS, none of them seemed to be complete. The another gene analysis of LNS, including the introns, are also necessary. This family of hereditary gout were clinically and enzymologically suggested as HPRT-Munich. But our blotting experiment with the restriction enzyme *Alu*I failed to show any mutations. Because *Alu*I yields small DNA

fragments, it is possible that distinct patterns of mutated locus might not be obtained. Therefore our cases could have whether HPRT-Munich or other new mutation of HPRT protein. Acknowledgement: pPR1, the cDNA clone of HPRT gene, was kindly gifted by Dr. Jolly.

II-58. Introduction of a Human β-Globin Gene into Mouse Germ Line: **Keiko MOMOI, Hideaki TOJO** (Inst. Lab. Anim. Sci., Toyama Med. Pharm. Univ., Toyama), **Yasuyuki FUKUMAKI** (Dept. Biochem., Sch. Med. Kyushu Univ., Fukuoka) **and Zen-ichi OGITA** (Dept. Pathol. Biochem., Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama)

The globin gene family provide an attractive model for the study of gene regulation in mammalian developmental process, as its expression is subject to tissue-specific and stage-specific regulation. To study the system of human β-globin gene expression we introduced that gene into mouse germ line by microinjection technique. Approximately 200 copies of the 4.4 kb *Pst*I fragment of human β-globin gene were microinjected into the male pronucleus of (C57BL/6×DBA/2) F_1 fertilized mouse egg. In this experiment, 25 mice were born and 4 mice (1 female and 3 males) carring human β-globin gene sequences were identified by dot-blot analysis of spleen DNA from these mice. Spleen DNAs from these 4 mice were digested either with *Eco*RI or *Bam*HI and the digests were analyzed by Southern-blotting hybridization. This analysis showed that one of 4 mice carried the entire sequence human β-globin gene. To investigate the inheritance of that gene, 4 transgenic mice were mated with normal C57BL/6 females and male. Among the offsprings, we observed a high rate of death (10 of 16 pups) on 10th and 15th day of birth, respectively. No firm data on explanation for this phenomenon have been obtained yet. Further detailed studies should be required for adequate explanation for these matter.

II-59. ヒト抗体 γ1鎖遺伝子のトランスジェニックマウスにおける発現：**山村研一・海老原妙子・紙野晃人・熊原雄一**（阪大・医・4 内），**工藤　明・渡辺　武**（佐賀医大・免疫）．**Gene Expression for Human Immunoglobulin γ1 Chain in Transgenic Mice:** K. YAMAMURA, T. EBIHARA, A. KAMINO, Y. KUMAHARA (4th Dept. Intern. Med., Osaka Univ. Sch. Med., Osaka), A. KUDO and T. WATANABE (Dept. Immunol., Saga Med. Sch., Saga)

トランスジェニックマウスの系は，単離した遺伝子の構造とその発現の組織特異性や時期特異性を正常細胞で解析できる系の一つである．そして遺伝子が発現した場合，その産物である蛋白の細胞内外における機能的役割ばかりでなく，その産物，その細胞への影響も直接解析できる．私たちは，単離したヒト抗体γ鎖遺伝子をマウス受精卵に導入し，これが組み込まれたトランスジェニックマウスを

得た．遺伝子発現の有無をみるために，各組織を固定し組織切片を作製し，蛍光抗体法にてヒトγ鎖の産生を解析したところ，脾臓でのみ発現が確認された．さらに，脾細胞をリポポリサッカライドで刺激するとヒトγ鎖産生細胞は増大するが，コンカナバリン A では不変であるところから，B リンパ球でのみ発現しており，分化増殖とともに正常な調節を受けて発現してくることがわかった．そして，このヒトγ鎖がマウス内因性 L 鎖と結合し，完全な IgG 分子を構成し，細胞に分泌してくることも明らかとなった．また，マウスの内因性抗体産生には何の影響も及ぼさず，ヒトγ鎖とマウスμ鎖もしくはγ鎖を同時に産生している細胞も存在していた．以上のことから，将来遺伝子治療が行われる際，導入予定の遺伝子が，この系を用い，どのように発現し，その効果を細胞内あるいは個体内で及ぼすのかを検討できることを示しえた．

II-60. ヒト培養細胞でのヒト遺伝子産物の大量発現：山田正夫（国立小児病院小児医療研究センター），**Terri Grodzicker (Cold Spring Harbor Laboratory). Mass Production of Human Gene Products in Human Cultured Cells:** M. YAMADA (Natl. Child. Med. Res. Cent., Tokyo) and T. GRODZICKER (Cold Spring Harbor Lab., USA)

[目的] 遺伝子工学の進歩により，ヒトの遺伝子が分子クローン化できるようになり，ヒトの遺伝病の〈治療〉が考えられるようになった．根本的治療に至るまでの間，一時的治療法として，変異に対応する正常の遺伝子産物を患者に投与することが考えられる．この場合，蛋白質は翻訳後に正しく修飾を受けたほうが体内での安定性および生物学的活性に有利なので，分子クローン化されたヒトの遺伝子をヒトの細胞で大量に発現できる系が望まれる．[方法] ヒトアデノウイルスはヒトの細胞に感染でき，感染後期には宿主細胞の蛋白合成を阻害し，合成される 90% 以上の蛋白質はウイルス由来であるなど，目的に合致している．そこで，アデノウイルスをベクターとして外来性の遺伝子産物を組織培養細胞で大量に作る系を開発した．単純ヘルペスウイルスのチミジンキナーゼ遺伝子 (TK) と，ヒトのじゅう毛性ゴナドトロピンの遺伝子 (hCG) を，アデノウイルスの主後期プロモーターの下流に *in vitro* と *in vivo* の DNA recombination を使い挿入した．[結果] 発現された TK 蛋白質は酵素活性を有し，hCG は正常な糖鎖を持ち，かつ分泌された．発現された TK 蛋白質は全細胞蛋白質の約 1% であり，他の組織培養細胞：ウイルスベクター系に比べて著しく高い発現を得た．

参考：*ProNAS* **82**: 3567–3571 (1985)

II-61. *In Vivo* Activation of Cellular Oncogenes in a Variety of Solid Tumors from Children: Kohnosuke MITANI (Dept. Maternal Child Health, Univ. Tokyo, Tokyo), **Yutaka NAKAHORI, Masao YAMADA, Noboru KOBAYASHI and Yasuo NAKAGOME** (Natl. Child. Med. Res. Cent., Tokyo)

It is well known that in human somatic cells there are cellular oncogenes (c-*onc*) which have homology with viral oncogenes (v-*onc*) detected in retrovirus. In some malignant tumors, amplification of certain c-*onc* has been disclosed. We have analyzed Southern and Northern blotting using a total of 11 different v-*onc* probes on samples resected from 34

solid tumors in children. Thirty-one were studied on DNA and five were performed on RNA. From these results, it was shown that two out of 16 DNA samples from neuroblastoma had 10–100 folds of amplification of N-*myc* oncogene. The possibility of several folds of c-*onc* amplification was also suggested in some other tumors and their studies are still in progress.

III-1. Genetic Polymorphism of the Seventh Component of Complement (C7)[1]: Typing by Agarose Gel Isoelectric Focusing and Immunoblotting: Hiroaki NISHIMUKAI and Yoshihiro TAMAKI (Dept. Forensic Med., Med. Coll. Oita, Oita)

Genetic polymorphism of C7 was studied by the methods of agarose gel isolectric focusing (pH 5–8) and immunoblotting. Serum or ACD-plasma samples from healthy Japanese individuals, living in the western part of Japan, were obtained. The samples were treated with neuraminidase prior to isoelectric focusing. In the Japanese subpopulation, C7 1, C7 2, C7 4, and a new cathodal variant tentatively named C7 5 were found. The results of family study provide supporting evidence for a codominant mode of inheritance with two alleles at a single autosomal locus. The allele frequencies for *C7*1, C7*2, C7*4*, and *C7*5* were 0.809, 0.104, 0.038, and 0.049, respectively. The observed phenotype numbers do not differ significantly from those expected under the assumption of a Hardy-Weinberg equilibrium. The linkage relationship between C7 and C6 loci was analyzed. The segregation data of C7 and C6 showed no discrepancy from the postulation of a linkage between these two loci. The association between C7 and C6 alleles was also analyzed, but no significant positive association was found.

1) Nishimukai, H. and Tamaki, Y. *Vox Sang.* in press.

III-2. 日本人における PLG 型の分布：免疫ブロッティング法による検出：山口眞由（近畿大・医・法医），**西向弘明**（大分医大・法医），**松井清司・松本秀雄**（大阪医大・法医）. **Polymorphism of Human Plasminogen in Japanese: Using IEF and Immunoblotting:** M. YAMAGUCHI (Dept. Legal Med., Kinki Univ., Sch. Med., Osaka), H. NISHIMUKAI (Dept. Legal Med., Oita Med. Coll., Oita), K. MATSUI and H. MATSUMOTO (Dept. Legal Med., Osaka Med. Sch., Takatsuki)

プラスミノーゲン (PLG) の変異型の検索は従来，免疫固定法やザイモグラム法で行ってきた．今回，ノイラミニダーゼ処理血漿を用いて等電点電気泳動を行い，免疫ブロッティング法で PLG パターンの検出を試みた．日本人の PLG 表現型は 12 種類に分類でき，その 90% は PLG1 であった．

家族試料の検査結果より，PLG 型は常染色体上の一つの座の優劣のない 1 対の対立遺伝子により規定されることが示された．健常人試料の検査の結果，日本人集団に少なくとも 8 種類の対立遺伝子 (1, 2, A, A2, A3, B, B2, C) の存在を認めた．おのおのの遺伝子頻度は *PLG**1=0.951, *PLG**2=0.016, *PLG**A* = 0.007, *PLG**B* = 0.014, *PLG**C* = 0.011, *PLG**(A2,A3,B2)* = 0.001 であった．A2,A3,B2 は，血漿をノイラミニダーゼ処理をしないと検出が困難である．*PLG**B* 成分は，活性をもたないのでザイモグラム法では検出されない．免疫ブロッティング法は，検出感度が非常によく明瞭なパターンが得られ，抗血清も少量ですむので今後免疫固定法に代わるべき方法と考える．なお，新しい分類法によると上記の *PLG**1*, *PLG**2*, *PLG**A*, *PLG**A2*, *PLG**B*, *PLG**C* はそれぞれ *PLG**A*, *PLG**B2*, *PLG**A3*, *PLG**A2*, *PLG**M5*, *PLG**B* となる．

Skoda, U. *et al. Vox Sang.* in press

III-3. Genetic Polymorphism of Coagulation Factor XIII B Subunit in the Japanese Population: Existence of *FXIII B*2* Allele in the Japanese Population and Description of Three New Rare Alleles: **Shigeki NAKAMURA, Osamu OHUE and Kazue ABE** (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Polyacrylamide gel isoelectric focusing (PAGIEF) of neuraminidase treated plasma samples with pH range 4.0–7.0 followed by an electroblotting and enzyme immunoassay was done for the further investigation of FXIII b. In 435 Japanese subjects PAGIEF patterns of FXIII B were classified into five common and three rare allotypes. From the reference typing with standard samples which were a gift of Dr. Board, these five common allotypes coincided with 3-3, 1-1, 3-1, 3-2 and 2-1, respectively, and it was indicated that *FXIII B*2* allele exists in the Japanese population as in Caucasian. The existence of *FXIII B*2* allele in the Japanese population also confirmed three allele model of Board (1980), *i.e.*, *FXIII B*1*, *FXIII B*2* and *FXIII B*3* as common alleles. In addition, three rare allotypes were detected and considered to be controlled by three rare alleles which were designated *FXIII B*13*, *FXIII B*14* and *FXIII*15*. Family data were in accordance with the Mendelian inheritance. The gene frequencies calculated from 435 Japanese subjects were *FXIII B*1*=0.2977, *FXIII B*2*=0.0184, *FXIII B*3*=0.6805, *FXIII B*13*=0.0011, *FXIII B*14*=0.0011 and *FXIII B*15*=0.0011.

III-4. Genetic Polymorphism of C81 (α-γ) in the Japanese Population: **Shigeki NAKAMURA, Osamu OHUE and Kazue ABE** (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Polyacrylamide gel isoelectric focusing (PAGIEF) at pH 3.5–9.5 followed by an electroblotting was done for the detection of C81 phenotypes in 448 unrelated Japanese plasma samples and 47 matings with 56 offsprings. After 3 hr PAGIEF at a constant power of

10 W with maximum voltage of 1,000 V and 50 min electroblotting at 400 mA in the electrode buffer consisting of 25 mM Tris–192 mM glycine/20% methanol, pH 8.3, C81 was detected by the use of mono-specific anti C81 serum and peroxidase conjugated anti immunoglobulin. In this study, 3.1 M urea was added in the gels in order to obtain the clear-cut bands of C81. Phenotypes of C81 were classified into three common and four rare patterns, and these were considered to be controlled by two common alleles, *C81*A* and *C81*B*, and three rare alleles which were tentatively designated *C81*A1J*, *C81*A2* for acidic variants and *C81*B1* for basic variant. The alleles of *C81*A2* and *C81*B1* are new rare alleles detected in the Japanese population, but *C81*A1J* might correspond to *C81*A1* in the former studies. Family data were in accordance with the Mendelian inheritance. The gene frequencies were estimated as C81*A=0.6228, C81*B=0.3672, C81*A1J=0.0078, C81*A2=0.0011 and C81*B1=0.0011. The gene frequencies of two common alleles are similar to those of other ethnic groups.

III-5. Salivary Amylase Variants in Some Non-Human Primates: Goichi ISHIMOTO, Hideaki UDA (Dept. Legal Med., Mie Univ. Sch. Med., TSU) **and Shunji GOTOH** (Primate Res. Inst., Kyoto Univ., Inuyama)

Using polyacrylamide gel electrophoresis and starch-iodine staining, we examined salivary amylase isozymes in several species of non-human primates, and found that an extensive polymorphism exists in macaque monkeys and also that detectable enzymes are not present in lower primate species. Saliva samples were obtained by drooling from anesthetized animals with pilocarpine stimulation. In 65 samples comprising 8 species of macaques, 5 presumed homozygous phenotypes could be classified. Their zymograms consisted of at least 3 bands decreasing in intensity toward the anode. The main component of the commonest had a mobility slightly slower than that of human SA2 and the others showed gradually slower mobilities. Four presumed heterozygous phenotypes, each possessing one of less common component and the commonest one, were also observed. Like other genetic markers species-specific distribution of the phenotypes were evident in the amylase polymorphism. A papio samples showed similar pattern to one of the macaque homozygous phenotype, and 2 patas monkeys and 1 green monkey had an identical pattern to each other, which was different from those of macaques. The amylases of the lower primates sofar examined, that is, 1 capuchin, 1 spider monkey, 4 night monkeys and 4 galagos, showed all obscure and smear-like patterns. They did not show any detectable component, even though concentrated samples were applied. The enzyme assay by dinitrosalicylic acid method indicated that these monkeys had approximately 1/1,000 or less in enzyme activity than those of higher primates.

III-6. 非計量多次元尺度法による蒙古系民族の遺伝的関係：河合尚樹・鈴木広一・伊藤重徳・松本秀雄（大阪医大・法医）. **Analysis of Genetic Relations among Mongolian Races by Non-Parametric Multidimensional Scaling:** N. KAWAI, H. SUZUKI, S. ITO and H. MATSUMOTO (Dept. Legal Med., Osaka Med. Sch., Takatsuki)

遺伝子頻度より 2 集団間の遺伝的距離は，根井の式により定義される．しかし N 個の集団から求められる距離は n (n−1)/2 個となり，しかも N 次元空間のベクトルであるから，その相互の関係を理解することは困難である．このような N 次元空間のベクトルをできるだけ相互の関係を保存するように，二次元平面上に投影するのが，多次元尺度法と呼ばれる方法である．多次元尺度法は 1962 年 Shepard により初めて紹介され，Kruskal (1964), Guttman (1968) らによって改良されたものである．多次元尺度法は，距離の数量的関係を保存しようとする計量多次元尺度法と，その距離の順位のみに着目して解を求める非計量多次元尺度法とに分けられる．計量多次元尺度法はわずかな差異を大きな差異として表現する欠点があるため，遺伝学領域では非計量多次元尺度法 (non-parametric multidimensional scaling) が用いられる．

われわれは今回，蒙古系民族の標識遺伝子である Gm ab^3st と Gm afb^1b^3 をもつ 31 集団について，Gm 遺伝子による遺伝的距離を計算し，これに基づいて非計量多次元尺度法により 31 集団の相互の関係を求めた．その結果，蒙古系民族は大きく南方型，北方型の二つのグループに分けることができる．日本民族は南方型でなく，明らかに北方型蒙古系民族に属すると判断できる．

III-7. Frequency of Gelatinous Drop-like Corneal Dystrophy in Japanese Population: Keiko FUJIKI, Atsushi KANAI and Akira NAKAJIMA (Dept. Ophthalmol., Juntendo Univ. Sch. Med., Tokyo)

Gelatinous drop-like corneal dystrophy, primary corneal amyloidosis is a rare familial disorder characterized by massive subepithelial deposits of amyloid fibrils, which has been more often reported in Japanese than in foreign literature since the first report by Nakaizumi (1914). The consanguinity among the parents of the cases is very high at the rate of 48% and the rate of the first cousin marriage is 39%. The present study estimated the gene frequency of gelatinous drop-like corneal dystrophy by Kimura's formulae (1958), using the rate of consanguinous marriages among 59 parents of the cases, in which 49 cases were reported from 1914 to 1981 and 10 cases were followed up at Juntendo University from 1964 to June, 1985. The results are as follows: The gene frequency was calculated to be $q=0.0050\pm0.0015$. Therefore, the carrier of the recessive gene is 1 in 100 persons in general population, the incidence of the disorder is 1 in 31,500, and 3,500 patients is considered to exist in Japanese population aged from 5 to 79.

III-8. A Case Report of 2p Partial Trisomy: Hideo TAKI, Yasushi YOSHIOKA, Masahisa FUNATO (Dept. Pediatr., Yodogawa Christian Hosp., Osaka), **Shizuhiro NIIHIRA and Hiroko FUJITA** (Dept. Sci. Living, Osaka City Univ., Osaka)

The patient was born in July, 1984 to a 30-year-old mother and a 34-year-old father, as the term product of uneventful pregnancy and the third child. Birth length and weight were 44.0 cm and 1,980 g, respectively. Her head was controlled at age 6 months. She was referred for heart murmur at age of 11 months. Her height was 67.1 cm (−2.4 SD), weight was 7.0 kg (−2.1 SD) and head circumferrence was 40.7 cm (−2.9 SD). Also noted were frontal bossing, hypertelorism, marked epicanthal folds, ptosis, external strabismus, abnormal low set ears, broad flat pug nose with triangular nares, wide philtrum, micrognathia and hyperextensibility of fingers and toes. Radiological findings were 11 ribs bilaterally, hepatic herniation through the Morgagni foramen, agenesis or hypogenesis of right kidney and delayed bone age. She set alone at age of 1 year 2 months. Her DQ (MCC) was estimated at 27. The karyotype of the patient as 46,XX,dir dup(2)(pter→p23::p25→qter). The parents were chromosomally normal. The clinical findings in our patient were similar to those described in previously reported cases of partial 2p trisomy and also to cases of partial duplication of the region 2p23→2pter.

III-9. A Case Report of Interstitial Deletion of the Long Arm of Chromosome 2: **Hideo TAKI, Miki TOKUHIRO, Hiroshi TAMAI, Seiichi SHIMADA, Masahisa FUNATO** (Dept. Pediatr., Yodogawa Christian Hosp., Osaka), **Yuuko KOKUI** (Dept. Pediatr., Osaka City Univ., Osaka) **and Hiroko FUJITA** (Dept. Sci. Living, Osaka City Univ., Osaka)

Few deletions of the long arm of chromosome 2 have been reported. We here report an additional case. The patient, a male, was the first born to a non-consanguinous mother and father aged 24 and 30 years, respectively. The pregnancy was normal and delivery at term was spontaneous. Birth weight was 2,740 g. The baby was referred for a number of congenital stigmata immediately after birth. Physical examination revealed a severe malformation syndrome, consisting of microcephaly, scaphocephaly, large forehead, hypertelorism, deeply set microphthalmia, an intermittent squint, bilateral ptosis, downward slant, low set malformed ears, high arched palate, micrognathia, tapering fingers, talipes valgus, undescending testis and imperforate anus. Radiological and histological studies revealed aganglionosis of intestine, Meckel's diverticulum and perforation of intestine. Cardiovascular examination showed PDA, which was repaired at 58 days. Neurological development was poor and he failed to thrive. At the age of 8 months, weight was 3,470 g, length 48.0 cm, and head circumference 33.0 cm. Death occurred at the age of 8 months after recurrent respiratory failure, urinary tract infection (*Candida*) and sepsis. The Karyotype of the child was 46,XY,del(2)(pter→p23::q31.1→qter). The chromosomes of both parents were normal.

III-10. 4q 部分トリソミー：臨床像と出生前診断例の剖検所見：林　研[1]・高橋　司[3]・江見信之[4]・浮田昌彦[4]・塩田浩平[2]・亀山順治[5]・夏山英一[6]（京大・[1]産婦人・[2]先天異常解析センター，倉敷中央病院・[3]中検・[4]産婦人・[5]小児，[6]夏山病院）. **4q Partial Trisomy: Clinical and Pathological Findings of a Fetus Aborted Artificially after Prenal Diagnosis:** K. HAYASHI *et al.* (Dept. Obstet. Gynec., Kyoto Univ. Sch. Med., Kyoto)

4q 部分トリソミーは稀な染色体異常であるが，今回われわれは，46,XX, t(4;9)(q31;p24) の転座保因者である妊婦の出生前診断で 4q31 より遠位部分のトリソミーを診断した．妊婦は 32 歳の 2 回経妊 1 回経産婦で，妊娠 18 週に出生前診断を実施したところ，胎児は 46,XX,der(9), t(4;9)(q31;p24) mat と第 1 子と同一の核型であることが判明した．流産胎児は，300 g と AFD であったが，超音波断層法で予測されたように，口唇口蓋裂，著明な頸部のリンパ浮腫が認められ，両眼隔離，幅広く低い鼻，口角の下がった口，顔面中央部の低形成などをともなう 4q 部分トリソミーに特有な顔貌を呈していた．口唇口蓋裂以外は，強度の精神運動発達遅延を呈している第 1 子にも認められる共通の異常所見である．なお，2 回目の妊娠は，妊娠 2 カ月で自然流産しているが，第 1 子および今回の妊娠経過中にはとくに異常は認められなかった．流産胎児の病理解剖所見を含めたその他の詳細について報告した．

III-11. A Case of "Cri du Chat" Syndrome Due to Paternal Pericentric Inversion: Kiyoshi MIYAZAKI, Tsutomu YAMANAKA (Cent. Hosp., Aichi Pref. Colony, Aichi) **and Keiko ASANO** (Tosei Public Hosp., Aichi)

The propositus was the second female infant born to normal parents. The mother was 28 years old and the father 35 when she was born. There was no history of prior spontaneous abortions, and her elder brother was phenotypically normal. The patient was born at full term after uneventful pregnancy. Birth weight was 1,700 g. She sucked poorly and cried weakly. She was admitted to our hospital at age of 9 days because of multiple dysmorphic features. At admission the following abnormalities were observed: microcephaly, round face, hypertelorism, antimongoloid slants, bilateral preauricular tags, micrognathia, broad flat nasal bridge, right sided lateral cleft-like extension of mouth, bilateral simian creases and anocutaneous fistula. The chest showed retraction. She was feeded by nasogastric tube and gained weight steadily. Since the age of two weeks she became cyanotic and retraction was exaggerated when she cried. At the age of 48 days she suddenly died. Cytogenetic studies using GTG and RBA methods on peripheral blood lymphocytes revealed that the patient's karyotype was 5p−. Her mother's karyotype was normal, while her father was found to have a pericentric inversion of chromosome 5: 46,XY,inv(5)(p13q35). We considered that the patient was the product of "recombination aneusomy" due to paternal pericentric inversion and the karyotype was interpreted as 46,XX,dup q,inv(5)(p13q35)pat, indicating partial monosomy 5p and partial trisomy 5q.

III-12. Epistasis of Chromosomal Abnormality in a Case Affected with 5p− Syndrome and 9p Trisomy Syndrome: Masami HORI, Eihiko TAKAHASHI, Keiji SUZUKI, Yasushi ITANI, Shuh OHDE, Tsutomu OHNO, Naoki NIITSU (Div. Neonatol., Saitama Child. Med. Cent., Iwatsuki) **and Yoshimitsu FUKUSHIMA** (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

The proposita, a 7-month-old girl, was the first product of a 23-year-old mother and a 25-year-old father. They were healthy and not consanguineous. The mother had no history of abortion. There was no remarkable episodes without IUGR in the course of pregnancy. She was born at 39 week's gestation by cesarean section because of fetal distress. Her birth weight was 1,640 g. When she was admitted, following anomalies were recognized: microcephaly, a round face, hypertelorism, epicanthus, down-ward slanting of palpebral fissures, a vertical wrinkle on the forehead, low-set ears, large dysplastic ears, micrognathia, a high-arched palate, a cat-like cry, a short neck, a sacral dimple, kyphosis, clinodactyly and a single flexion crease of 5th fingers, hypoplastic nails, knock knees, congenital heart disease (extreme teratology of Fallot). Roentgenography of bones showed delayed ossification of pubic bones and hypoplastic meso- and telephlangea. High-resolution chromosome analyses revealed that the karyotype of the patient was 46,XX,−5,+der (5),t(5;9)(p13.3;p21)mat, and that the karyotype of the mother was 46,XX,t(5;9)(p13.3;p21). The karyotype of the father was normal. The patient suffered from 5p− syndrome and 9p trisomy syndrome simultaneously. She had both clinical features of 5p− syndrome and of 9p trisomy syndrome. Epistasis (a dominant phenotypic effect of chromosome abnormality in a case with two or more chromosome aberrations) is not recognized.

III-13. Partial Trisomy 12q Due to Balanced Maternal 9;12 Translocation: Mitsuo MASUNO, Yoshimitsu FUKUSHIMA, Yoshitsugu SUGIO and Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

Partial trisomy 12q is characterized by mental retardation, dolichocephaly or brachycephaly, hypertelorism, flat nasal bridge, low set ears, poor lobulation, down turned mouth, micrognathia or pointed chin, short neck, loose skin at nape, wide set nipples, simian creases, genitourinary anomalies, sacrococcygeal dimple and heart defect. A three-year-old boy with partial trisomy 12q,46,XY,der(9),t(9;12)(q34.3;q24.31)mat is described. To our best knowledge, this is the second case in Japan. The proband was the first product of 28-year-old mother and 30-year-old father, who were unrelated. Because of placental dysfunction, caesarean section was performed at 40 weeks of gestation. His birth weight was 2,055 g. The early neonatal period was complicated by hypoglycemia, hyperbilirubinemia and feeding difficulties. He had also retinopathy due to prematurity. Main clinical fea-

tures were severe mental retardation (DQ 23), microcephaly, brachycephaly, flat facial profile, hypertelorism, antimongoloid slant, ptosis, broad eyebrows, flat nasal bridge, anteverted nares, long philtrum, carp shaped mouth, pointed chin, short tapering fingers and overlapping toes. Dermatoglyphics was abnormal : low TFRC (47) and absent C digital triradii. Arachnoid cyst at the left middle cranial fossa, mild dilatation of the lateral ventricles and asymmetrical Sylvian fissures were noticed by CT scan.

II-14. Beckwith-Wiedemann Syndrome and 11p Trisomy: Marie NISHIHARA, Takashi YAMAIRI, Yoshiyuki OKANO, Yukinobu OSASA, Hiroko YAMAMOTO, Yutaka HASE, Tsuneo TSURUHARA (Child. Med. Cent. Osaka City, Osaka) **and Hiroko FUJITA** (Dept. Child Health, Sci. Living Fac., Osaka City Univ., Osaka)

Beckwith-Wiedemann syndrome (BWS) and 11p trisomy syndrome have common clinical features such as macroglossia, umbilical hernia and macrosomia. We experienced in each case. 1) Case 1: This female infant was born to healthy and unrelated parents with uneventful 37-week gestation. Her birth weight was 4,990 g and length was 51.3 cm. She was the second child of a 25-year-old mother and father. Her sister was healthy. BWS was suspected because of large birth weight, macroglossia, ear lobe creases, nevus flammeus umbilical hernia, hemihypertrophy, hapatomegaly and nephromegaly. Hypoglycemia, hyperinsulinemia and tumor were not demonstrated. The chromosomal analysis showed a normal karyotype, 46,XX. 2) Case 2: This case had been reported in 1980 by Okano *et al.* in the Annual Meeting of this Society. This female infant was born at the 39th week with asphyxia, as the second child of healthy and unrelated parents. Her brother was healthy. The birth weight was 3,100 g. Physical examination revealed macroglossia, umbilical hernia, hypotonia, soft and wrinkled skin, dysmorphic face, hepatosplenomegaly, intestinal malrotation and Meckel's diverticulum. These features were similar to those of BWS. The karyotype of this patient was 46,XX,$-4,+$der(4),t(4;11)(q35;p13)pat.

BWS is characterized by macrosomia, macroglossia and umbilical anomaly. More than 50 cases with BWS have been reported in Japan, which have such trias. Chromosomal studies were performed on 12 cases and they indicated a normal karyotype. The reported cases of 11p trisomy (patients with 11p15 trisomy) have the trias of BWS and mental retardation which will be a characteristic feature of 11p trisomy. Since 11p trisomy has typical features of BWS, chromosomal analysis should be performed in BWS.

III-15. A Case of an Interstitial Deletion of the Short Arm of Chromosome 17: **Mashio KITATANI,[1] Hideaki CHIYO,[1] Hiroko KAWASHIMA,[1] Mamoru OZAKI,[1] Makoto AWAKURA,[2] Koichi TOYOTA,[2] Masato MORITA,[2] Keiso TACHI,[2] Toshio ASAI[2] and Shoichiro SHIKE[2]** ([1]Dept. Clin. Genet., Inst. Hum. Genet., [2]Dept. Pediatr., Kanazawa Med. Univ., Ishikawa)

Interstitial deletion of the short arm of chromosome 17 is extremely rare. To our knowledge, only two cases have been reported. The patient, two years old male, was born after uneventful pregnancy. His birth weight was 3,080 g, length was 49 cm in 41 weeks gestation. The mother was 29 years old and the father was 33 years old at his birth. He was admitted because of congenital anomalies and congenital heart disease. The following abnormalities were observed: flattened face, narrow and upslanting palpebral fissures, epicanthal folds, flat nasal bridge, low-set ears, highly arched palate, micrognathia, short fingers, syndactyly of 2/3 toes and ventricular septal defect. His dermatoglyphics inspection revealed high frequencies of interdigital paterns and traversed main lines. Growth retardation and psychomoter retardation were observed. On physical examinations at 21 months, his weight was 8,870 g (−2.2 SD), height was 78.8 cm (−1.5 SD) and his developmental quotient was 47. Cytogenetic studies using high-resolution banding techniques in peripheral lymphocyte revealed a karyotype of 46,XY,inv(9),del(17)(p11.2p11.2). The karyotypes of his parents were 46,XX,inv(9) and 46,XY, respectively. Miller-Dieker syndrome in monosomy of distal 17p was discussed previously, but hypertonicity or seizure was not observed in this case.

III-16. 母親の転座染色体，t(8;22)(p23;q11) に由来した 22 番染色体部分モノソミーの姉妹例：藤原正貴・安積順一・塩野　寛（札幌医大・法医）. **Two Sisters of Partial Monosomy 22 Resulting from Maternal Translocation, t(8;22)(p23;q11):** Masataka FUJIWARA, Jun-ichi AZUMI and Hiroshi SHIONO (Dept. Legal Med., Sapporo Med. Coll., Sapporo)

長女は，昭和 57 年 3 月 10 日，在胎 39 週で出生．仮死認め，12 日間保育器を使用．生下時体重 2,400 g，身長 48 cm．精神発達遅延傾向あり，頸定 4 カ月，お座り 7 カ月，つかまり立ち 1 歳 7 カ月，一人歩き 1 歳 11 カ月．いまだ意味のある言葉は言えないが，2〜3 語文は理解しているようすである．生下時より心雑音を認め，満 1 歳時総動脈管症の診断で開胸術試みるも，治療不可能と判定されそのままになっている．チアノーゼ強度で太鼓バチ指を認めた．顔貌は正常であるが内眼角贅皮を認めた．次女は，昭和 59 年 9 月 13 日，在胎 33 週，生下時体重 1,650 g，身長 39 cm で出生．仮死（−）．生下時に，先天性小腸閉塞，多指症，耳介奇形を認めた．さらに心雑音を認め，先天性心疾患が疑われる．生後 4 カ月で退院後，難治性下痢症で体重増加が不良で，易感染性もあり，入退院を繰り返す．神経学的には頸定 5 カ月，お座り，つかまり立ちは不可と遅延傾向がみられる．言葉は現在，喃語程度である．両親には外見上特記すべき異状は認められない．母親は，長女出産の

前に一度自然流産（4カ月）の既往がある．染色体検査の結果，父親は正常，母親は 46,XX,t(8;22)(p23;q11)，姉妹は 45,XX,−8,−22,der(8),t(8;22)(p23;q11)mat であった．

III-17. A Case of 18 Trisomy Mosaicism with Pericentric Inversion of Chromosome No. 4: **Kenji NARITOMI, Chuken MIYAGI and Kiyotake HIRAYAMA** (Dept. Pediatr., Univ. Ryukyu, Okinawa)

An 18 trisomy mosaicism with pericentric inversion of chromosome no. 4 was found in a patient with mild combined phenotypes of 4p monosomy and 18 trisomy. The propositus is a 57-day-old boy born to a 34-year-old primipara primigravida mother and a 36-year-old healthy father. The parents are not related. The mother conceived him after artificial induction of ovulation because of infertility. She was treated for threatened abortion at the second months of gestation. He was delivered at 39th week by cesarean section because of fetal distress. His birth weight was 2,064 g and Apgar score was 6. Cloudiness of amniotic fluid was pointed out. Cyanosis due to dyspnea developed soon after birth, and mechanical ventilation was indicated. He had small stature and poor nutrition. The head was micro- and brachycephalic with wide anterior fontanel. He had a round scalp defect on his occiput. The forehead was high and bossing. The glabella was prominent with capillary hemangioma in the middle portion. The eyebrows were sparse toward interior. The palpebral fissures were slanted downward. He had a saddle nose. The corner of the mouth was downturned and its opening was small. The chin was small and slightly receded. The ears were slightly low-set. In the trunk, he had mild short sternum, hepatomegaly and bilateral inguinal hernia. In the extremities, his left forearm was short with severe radial and mild ulnar hypoplasia and hypoplasia of left thumb. He had camptodactylia of left second to fifth fingers with no second flexion crease. Two arch patterns were found. Chromosomal analysis revealed 46,XY,inv(4) and 18 trisomy mosaicism (2 : 1). Inv(4) was also found in his father. The breakpoints were defined as p16 and q21.1 by high resolution banding (GTG, RHG). The presence of phenotypes similar to 4p monosomy was supposed to be due to a partial deletion at 4p16. It may be related to the concurrent 18 trisomy mosaicism, because his father, an inv(4) carrier, is phenotypically normal.

III-18. A Newborn with Noonan-like Phenotype and an Extrachromosome der(22),t(11;22)(q23.3;q11.23): **Tomoko HASEGAWA** (Dept. Genet., Shizuoka Child. Hosp., Shizuoka), **Tsunehiro YOKOCHI** (Clin. Pathol., Shizuoka Child. Hosp., Shizuoka), **Ken UEDA** (Dept. Cardiol., Shizuoka Child. Hosp., Shizuoka) **and Shinichi NIIJIMA** (Neonatal Cent., Izunagaoka Branch Hosp., Juntendo Univ., Shizuoka)

Making diagnosis of malformed newborns or children in early infancy is much more

difficult than when the children are grown up. A case of a malformed male newborn was presented here, who showed partial trisomies of chromosomes 11 and 22. The proband was a 2,620 g male born to a 36-year-old healthy mother and 34-year-old healthy father at 40 weeks' gestation by spontaneous vaginal delivery. The newborn had a prominent webbed neck and heart murmur, and was clinically diagnosed as Noonan syndrome. The face had a peculiar appearance with a short broad nose and long philtrum. The ears were large, low set, slightly malformed with preauricular tags and rotated posteriorly. Accurate cardiological examination revealed that the congenital heart condition was atrial septal defect. Cytogenetic studies were performed because of the findings atypical of the Noonan syndrome and revealed an extra small chromosome similar to 22q−. Chromosome examination of the parents demonstrated a t(11;22)(q23.3;q11.23) in the mother. The patient's karyotype was therefore described as 47,XY,+der(22),t(11;22)(q23.3;q11.23)mat. After obtaining the cytogenetic results, we were able to prepare an early educational program for the child including genetic counseling to the parents.

III-19. A Study of Abnormal Histogenesis in Fetuses with Chromosomal Abnormalities:

S. MIYABARA (Dept. Pathol., Saga Med. Sch., Saga), **K. SUZUMORI** (Dept. Obstet. Gynec., Nagoya City Univ. Med. Sch., Nagoya), **N. SAITO** (Dept. Obstet. Gynec., Kyushu Rosai Hosp., Kitakyushu), **M. MATSUMOTO** (Dept. Obstet. Gynec., Osaka Perinat. Cent., Osaka), **T. IKENOUE** (Dept. Obstet. Gynec., Kagoshima Municip. Hosp., Kagoshima) **and K. SHIOTA** (Cong. Anom. Res. Cent., Fac. Med., Kyoto Univ., Kyoto)

For the investigation of abnormal histogenesis, which is supposed to occur in fetuses with chromosomal abnormalities, organs of fetuses aborted after prenatal diagnosis by amniocentesis were fetopathologically examined. A total of eleven fetuses (4 cases of trisomy 21, 3 cases of trisomy 18, 2 cases of 47,XXY, 2 cases of partial trisomy) have been analyzed since Nov. 1983. Characteristic abnormalities were found in the following two cases of partial trisomy. Case 1. Partial trisomy 2p fetus. Male, 21 weeks, B.W.: 360 g. Chromosome: 46,XY,der(16),t(2;16)(p23;p13)mat. Cystic spaces were found in cut surfaces of both adrenals. Microscopically, many clusters consisting of neuroblasts proliferated on cystic walls. *Diagnosis: Neuroblastoma.* Case 2. Partial trisomy 6q fetus. Male, 19 weeks, B.W.: 385 g. Chromosome: 46,XY,der(7),t(6;7)(q21;p22)mat. Microscopically, there were localized nephrogenic tissues corresponding to macroscopical irregular nodules on the surface of both kidneys. Many microcysts were present in cortex. *Diagnosis: Renal dysplasia.*

It is noteworthy that there is also a report on an infant case of partial trisomy 2p with neuroblastoma involving chromosomes 2 and 16 (Nagano *et al.*, 1980). It would be further

meaningful to indicate that the breakpoint 2p23 of the present fetus corresponds to the locus (2p23-24) were N-*myc* is localized (Schwab *et al.*, 1984).

III-20. High-Resolution Banding Analysis of Human Chromosomes by a Simple Addition of Ethidium Bromide to Bone Marrow Cultures: **Shigeo HORIIKE, Shinichi MISAWA, Masafumi TANIWAKI, Shohei YOKOTA, Hiromi YASHIGE, Johji INAZAWA, Tsukasa OKUDA, Tatsuo ABE and Tatsuro TAKINO** (3rd Dept. Inter. Med., Kyoto Pref. Univ. Med., Kyoto)

A considerable number of processing methods have been reported for obtaining prometaphase or early metaphase cells. Among them, DNA-binding chemicals are known to elongate chromosomes by simple addition into the culture. We undertook several experiments to determine the conditions that allow us to obtain a large number of early mitotic cells in the culture of bone marrow cells from patients with hematological malignancies by introducing ethidium bromide (EBr) to the culture. Prefixation treatment of cultured human bone marrow cells with EBr induced a dose- and time-related elongation of chromosomes. When compared with EBr-free cultures, a 2.9-fold increase in the yield of early mitotic cells with more than 400 bands per haploid set of chromosomes was achieved by simply adding 10 μg/ml of EBr during the last 2 hr of culture. The proportion of early mitotic cells was equal to that obtained in MTX-synchronized cultures. By application of this method we identified break points of pericentric inversion of chromosome 16 in acute myelomonocytic leukemia with dysplastic bone marrow eosinophilia to be 16p13.12 and 16q22.1 and those of the Ph^1 translocation in chronic myelogenous leukemia to be 9q34.1, and 22q11.2.

III-21. Common Fragile Sites in Folate-Free Medium with 5-Bromodeoxyuridine: **Akira KUWANO, Ichiro MURANO and Tadashi KAJII** (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Peripheral blood lymphocytes were cultured for 96 hr from seven normal individuals, three males and four females, and three fra(X)-positive males, using the following culture conditions. 1) MEM+BrdU: Eagle's minimum essential medium with 5-bromodeoxyuridine added at a final concentration of 20 mg/liter for the last 6 hr. 2) MEM-FA: MEM without folic acid (Nissui). 3) MEM-FA+BrdU: 5-bromodeoxyuridine was added to MEM-FA at a final concentration of 20 mg/liter for the last 6 hr. All culture media were supplemented with 5% fetal calf serum. Conventional Giemsa-stained chromosome slides were prepared, and 100 metaphases in each condition were screened for gaps and breaks. The slides were then destained, Giemsa banded, and the localization of gaps and breaks was

determined. The mean rate of the common fragile sites observed was 27.1% (range 6% to 59%) with MEM+BrdU, 28% (13% to 52%) with MEM-FA and 82.3% (21% to 136%) with MEM-FA+BrdU. The effect of the folate-free medium and the addition of BrdU was thus synergistic. Three common fragile sites, 18q12, 7p14 and 2p24, were induced with MEM-FA+BrdU. These sites have been known to be induced by caffeine+fluorodeoxyuridine, but not by BrdU. The rates of fra(X) in three fra(X)-positive males were all 4% with MEM-FA, while they were all 2% with MEM-FA+BrdU. The addition of BrdU thus worked to decrease the rates of fra(X).

III-22. Studies on Characterization and Staining Affinities of Acridine and Quinoline Derivatives to Human Chromosomes: Kouichi MAMBA, Misako GOMI, Mutsuo KITAHAMA (Dept. Legal Med., St. Marianna Univ. Sch. Med., Kawasaki) **and Akira UCHIUMI** (Natl. Chem. Lab. Indust., Tsukuba)

The present study was carried out to examine the characterization and staining affinities of three acridine and four quinoline derivatives as new fluorescent dyes to human chromosome samples. These three acridine derivatives synthesized are bis-[9-(2-methoxy-6-chloro acridyl)]-1,4-phenyl dihydrazone, bis(9-acridyl)-9,10-anthryl dihydrazone, and bis[9-(2-methoxy acridyl)]-1,4-phenyl dihydrazone. While the four quinoline derivatives synthesized are bis(2-quinolyl)-1,4-phenyl dihydrazone, bis(2-quinolyl)-1,4-anthryl dihydrazone, bis(4-quinolyl)-1,4-phenyl dihydrazone, and bis(4-quinolyl)-9,10-anthryl dihydrazone. The following results were obtained: 1) The three acridine and four quinoline derivatives showed fine staining affinities to the chromosome samples. 2) The fluorescent colors of the three acridine derivatives showed yellow or orange, and the absorption maximum values were between 441 and 509. 3) The fluorescent colors of the four quinoline derivatives showed yellow or yellowish-green and the absorption maximum values were between 351 and 429. 4) The dye pair bis(9-acridyl)-9,10-anthryl dihydrazone in the acridine derivatives/methyl green produces a weak banding pattern near the centromeric position of a pair of chromosomes in A and C groups. 5) The dye pair bis(2-quinolyl)-9,10-anthryl dihydrazone or bis(4-quinolyl)-9,10-anthryl dihydrazone in the quinoline derivatives/methyl green produces a weak banding pattern near the centromeric position of a pair of chromosomes in A and C groups.

III-23. An Improved Method for Ascertainment of Parental Origin of Chromosomes: Tsutomu KAMEI, Sei OKIMOTO, Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ., Nagasaki) **and Midori SOHDA** (RERF, Nagasaki)

We developed an improved method, a high-resolution dual Q-R banding technique, for

the ascertainment of the parental origin of a chromosome abnormality in a child. This method increases the ascertainment rate. *Materials and method:* prometaphase (550–850 band-stage) plates of cultured peripheral blood lymphocytes (Ikeuchi and Sasaki, 1979) from two sets of parents and a child were QFQ- and RFA-banded sequentially (Niikawa and Kajii, 1975). As controls, metaphase plates of the two families were banded with the same method. Transmission of heteromorphic chromosomes 3, 4, 13, 14, 15, 21 and 22 from parents to child was traced on both the prometaphase and the metaphase plates. *Results and comment: Family 1:* Out of the 14 (7 pairs) different heteromorphic chromosomes, 13 chromosomes of the child were identified for their parental origin, when prometaphase spreads were used. In metaphase spreads, however, the derivation was ascertained in 5 different chromosomes. *Family 2:* The origin of the child's chromosomes was determined in 13 different chromosomes at prometaphase, but in only 8 chromosomes at metaphase stage. The almost complete ascertainment rate when using the high-resolution dual Q-R banding method is due to its enhancement property on the heteromorphisms such as the size of satellites, satellite stalks and short arms as well as fluorescence markers on them. Applications of this method may include (1) determination of the origin of chromosome abnormalities, (2) parent identification, (3) determination of twin zygosity, and (4) determination of mosaicism and/or chimerism.

III-24. Comparison of Chromosome Aberrations by Ordinary Staining and G-Staining Methods in Hiroshima A-Bomb Survivors: Kazuo OHTAKI, Hachiro SHIMBA and Akio AWA (Dept. Genet., RERF, Hiroshima)

Radiation-induced chromosome aberrations are known to persist in the circulating lymphocytes of A-bomb survivors. The frequency of these aberrant cells proved to be proportional to the estimated dose received by each individual. The majority of chromosome aberrations were of the stable type. It is difficult by ordinary stain method (O-method) to detect any abnormalities associated with subtle changes of chromosome length or the position of centromere, while such aberrations can be identified objectively by G-banding method (G-method). In the present study a total of 4,175 cells from 40 Hiroshima A-bomb survivors were analyzed by both methods. The frequency of aberrant cells was 20.4% by the O-method, and 24.6% by the G-method. Of them 232 cells were detectable only by the G-method, with 252 aberrations including translocations, inversions, deletions and complex intra- and inter-chromosome exchanges. A total of 1,023 aberrations were found by the O-method, while 1,284 aberrations were detectable by the G-method. Complex chromosomal exchanges involving three or more breaks were observed in 126 out of 1,284 aberrations. The number of chromosomes participating in the exchange aberrations was 1,536 by the O-method, and 2,206 by the G-method, respectively (G : O = 1 : 0.70).

The frequencies of aberrant chromosomes identified by the G-method increase with radiation doses more sharply than those by the O-method.

III-25. 産婦人科における X chromatin の応用：立崎達夫・佐藤重美・高野　敦（弘前大・産婦人），**斉藤　勝**（弘前市立病院・産婦人）. **A Use of the X Chromatin in the Field of Obstetrics and Gynecology:** T. TACHIZAKI, S. SATOH, A. TAKANO (Obstet. Gynec., Hirosaki Univ., Hirosaki) and M. SAITOH (Obstet. Gynec., Hirosaki City Hosp., Hirosaki)

[研究方法] cervical scraping smear, Papanicolaou 染色したものを用い，X chromatin 出現率 (BBF: barr body frequency) を百分率で算出した．対象は，妊娠初期群 20 例，流産群 22 例，閉経後群 20 例，不妊症群 13 例（同一月経周期の月経時と排卵前期），子宮頸癌Ⅱ・Ⅲ期 5 年生存群 30 例，非生存群 18 例，子宮体癌 5 年生存群 18 例，非生存群 10 例であった．[結果] 1) estrogen との関係：妊娠初期群の平均 BBF は 17.0%，流産群 8.1%，閉経後群 6.6%，月経時平均 8.3% が排卵前期平均 19.4% に増加した．2) 子宮頸癌 5 年生存群平均 17.6%，非生存群平均 11.1%，子宮体癌 5 年生存平均 17.9%，非生存群平均 8.8% であった．妊娠初期群と流産群 ($p<0.01$)，妊娠初期群と閉経後群 ($p<0.01$)，月経時と排卵前期 ($p<0.05$) と有意差を認めた．また子宮頸癌 5 年生存群と非生存群 ($p<0.01$)，子宮体癌 5年生存群と非生存群 ($p<0.01$) と有意差を認めた．[結論] estrogen は，BBF を増加させるものと考えられる．子宮頸癌，体癌において，BBF が高いほど予後良好と思われる．

III-26. Chromosomal Fragile Sites in a Japanese Population: Motoi MURATA (Div. Epidem., Chiba Cancer Cent., Chiba), **Ei-ichi TAKAHASHI and Tada-aki HORI** (Div. Genet., Natl. Inst. Radiol. Sci., Chiba)

Fragile sites (FS) are the specific points of chromosomes where isochromatid gaps are frequently observed under specific culture conditions. They have recently become of much interest because of their possible association with some clinical conditions, including mental retardation and cancer. It is, we conceive, inevitable to know their frequency in the general population before we shall investigate the probability of the disease association. In this study we tested various heritable FS manifesting in the cultured peripheral lymphocytes from healthy donors under three kinds of already established culture conditions: folic acid deprivation, distamycin A addition, and BrdU addition. Cases of FS so far detected are as follows: folate-sensitive fra(11)(q13) and fra(17)(p12), distamycin A-induced fra(16)(q22) and fra(8)(q24), and BrdU-requiring fra(10)(q25). Incidences are 1/456, 1/456, 5/264, 1/264 and 2/465, respectively. As a whole, the incidence of FS in a healthy Japanese population was estimated at approximately three in 100. Expressivity of constitutional FS was also examined and its distribution among individuals appeared to deviate from that expected

from random variation. Furthermore we proceeded a preliminary study on patients with leukemia. In one of three patients of acute myelomonocytic leukemia (M4 by FAB classification) who showed a marker inv(16)(p13;q22) in lymphoblastic cells, fra(16)(q22) was detected in lymphocytes with a very low expressivity.

III-27. 習慣性流産を主訴として発見された転座保因者群の妊娠歴：林　研[1]，川内幸恵[2]，江見信之[3]，浮田昌彦[3] ([1]京大・産婦人，倉敷中央病院・[2]中検・[3]産婦人)．
Analysis of Pregnancy in Carriers with Autosomal Translocation Whose Chief Complaint Is Repeated Abortion: K. HAYASHI *et al.* (Dept. Obstet. Gynec., Kyoto Univ. Sch. Med., Kyoto)

染色体の数的あるいは構造異常による遺伝子の量的，質的不均衡をともなう受精卵の大半は，発生初期に成長および分化の停止や異常を生じ，妊娠継続が不可能となり自然流産する．相互転座保因者の配偶子は，染色体異常をともなう頻度が高く，したがって反復流産する可能性が強い．転座保因の有無を確認することは，流産の原因を解明する一助となるのみならず，出生前診断上も重要である．そのため，われわれは習慣性流産を主訴とする夫婦を一単位として細胞遺伝学的に検討をおこなっている．方法は，スクリーニングには，末梢血培養で作製した標本を，全例 G-分染法および Q-分染法で分析し，異常例はそのうえ必要に応じ高分染法を含む他の分染法を併用した．夫婦単位で分析した症例は 211 例で，そのうち 8.5% に相当する 18 例に異常が発見されている．転座保因者群の総妊娠数は 78 で，そのうち 10 例に出生前診断を実施した．その結果は，3 例が不均衡型染色体異常，4 例が均衡型転座を呈しており，残りは正常であった．総自然流産数は 65 で，分娩数は出生前診断例のうち不均衡型転座以外の 7 例を含む 13 例である．それぞれの核型，妊娠歴，その他の臨床データーを報告した．

III-28. Age Dependent Changes in the Frequencies of Ara C-Induced Chromosome-Type Aberrations in Human Peripheral Lymphocytes: Kunikazu KISHI (Dept. Adult Health Sci., Kyorin Univ., Tokyo) **and Akira HONMA** (Tokyo Metropol. Inst. Gerontol., Tokyo)

Age dependent changes in the frequencies of chromosome aberrations in human peripheral blood lymphocytes can be considered to reflect DNA lesions or capacities of DNA repair or DNA synthesis of cells. Recently, it has been suggested that inhibition of DNA repair synthesis in the G1 phase of the cell cycle would be concerned in the induction of chromosome-type aberrations, such as dicentrics or rings. Though the mechanism of induction of chromosome-type aberrations by repair inhibitors has so far been unknown, we tried to investigate whether any informations on the age-related changes in DNA repair could be detected using 1-D-arabinofuranosyl cytosine (Ara C), one of inhibitors of DNA repair. Lymphocytes from 40 donors aged from 0 to 89 were cultured in the presence of 10 M Ara C

for 18 hr after the culture initiation followed by incubation in the medium containing 30 M bromodeoxyuridine for 42 to 47 hr. Dicentric (dic) and ring chromosomes were scored for the first mitoses after the culture initiation. Frequencies of dic and ring were shown to decrease with the ages of donors, and to follow the equation $y=77.3-36.08*\log x$, where y was the number of dic+ring per 100 cells and x was the age of blood donor (SD of reg. coeff., 3.15; d.f., 38; $p<0.001$). This phenomenon was considered to reflect certain changes of DNA lesions or repair capacities according to ageing, but further investigations including the mechanism of aberration induction by Ara C should be needed for the accurate interpretations for the present findings.

III-29. 羊水染色体検査でのモザイク症例について：木下芳広（慶応健康相談センター），**田村昭蔵**（慶応大・医・産婦人）．**Chromosome Mosaicism in Amniotic Fluid Cell Cultures in Keio Group:** Yoshihiro KINOSHITA (Keio Health Counseling Cent., Tokyo) and Shozo TAMURA (Dept. Obstet. Gynec. Keio Univ., Tokyo)

1972 年 11 月から 1985 年 9 月までの 970 例の羊水染色体検査（若干の羊水過多症等の研究例を含む）で，Level I mosaicism (single abnormal cell) が 37 例 (3.8%)，Level II mosaicism (pseudomosaicism) が 6 例 (0.62%)，および Level III mosaicism (true mosaicism) が 1 例認められた．これらの出現頻度は，欧米のものとほぼ同じであった．Level I mosaicism の欧米での報告では No. 2 トリソミーが多いが，われわれはこれを認めていない．その他の染色体異常の分布についてはほぼ同じである．Level II mosaicism はトリソミーモザイクの 5 例 (C, C, No. 11 (頭胸結合体)，No. 18, および No. 21) と母体細胞混入と思われる 46,XY/46,XX の 1 例であった．Level III mosaicism の 1 例は 45,X/46XX であったが，中絶後の胎児血および皮膚からともに 45,X/46,XY/47,XYY が認められた．羊水中の 46,XX の起源は不明である．常染色体の数的モザイクの文献例では No. 20 が一番多い．これは表現型の異常例が少なく，追跡調査でも確認されることが少ない．No. 20 トリソミーモザイクは，羊水染色体モザイクに特有のものとしてわれわれも今後十分留意すべきであろう．

III-30. An Autosomal Dominant Microcephaly with Dysmorphic Face: Hiroko KAWASHIMA, Mashio KITATANI, Hideaki CHIYO (Clin. Genet. Unit, Inst. Hum. Genet., Kanazawa Med. Coll., Uchinada) **and Naruhito TSUJI** (Ishikawa Handicapped Child. Hosp., Ishikawa)

A mother and son were found to have an autosomal dominant microcephaly with a characteristic dysmorphic face. Prominent manifestations include microcephaly, asymmetric face, prominent glabella, upslanted palpebral fissures, low-set cup-shaped ears, thick protruding lower lip, micrognathia and mild mental retardation. We believe that these patients have a previously undescribed type of autosomal dominant microcephaly which could not have been diagnosed without the mother's photographs in childhood. Such photographs are an essential tool in the recognition of this syndrome.

III-31. 口蓋裂，心室中隔欠損症，耳介奇形，停留睾丸を合併した3例：下澤伸行・石井尚吾・家島 厚（鳥取大・医・脳研・神経小児）. **Three Cases with Cleft Palate, Ventricular Septal Defect, Ear Anomalies, and Cryptorchidism:** N. SHIMOZAWA, S. ISHII, A. IESHIMA (Div. Child Neurol., Inst. Neurol. Sci., Tottori Univ. Sch. Med., Yonago)

口蓋裂，心室中隔欠損症 (VSD)，耳介奇形，停留睾丸（男児）を合併した3例を経験したので報告した．一般に口蓋裂，心室中隔欠損は多因子遺伝に支配され，奇形のなかでも特異性は少なく，症候群の主要構成要素としての可能性は少ないと思われる．しかし Shprintzen らは，口蓋裂，VSD に特徴的な顔貌を呈し，軽度の知能障害を呈した症例を集め Velo-Cardio-Facial 症候群として報告している．今回の3症例を含め過去15年間に当科外来を受診した7,200余名のうち，口蓋裂に心奇形を合併した症例は8例あった．その内訳は，染色体異常が3例 (5p トリソミー，4q モノソミー，9q トリソミー)，残り5例のうち VSD を合併している3例が今回の症例である．3症例は，口蓋裂，VSD 以外に成長障害，顔貌の特徴として耳介奇形，小さい鼻，両眼隔離，また男児に停留睾丸を認め，他にもいくつかの共通する特徴をもっている．このことより，特異的な奇形はないが，臨床症状の組み合わせより一症候群としての可能性を顔写真を呈示して検討した．また鑑別疾患として Shprintzen syndrome CHARGE association をあげ，その臨床像を比較した．

III-32. CHARGE Association の2例：神村直久・西村悟子・家島 厚（鳥取大・医・脳研・神経小児）. **Two Cases with CHARGE Association:** N. KAMIMURA, S. NISHIMURA and A. IESHIMA (Div. Child Neurol., Inst. Neurol. Sci., Tottori Univ. Sch. Med., Yonago)

CHARGE Association の2例を報告した．症例1は，左網脈絡膜欠損・小眼球症，難聴を伴う小耳症，成長障害，精神運動発達遅滞があり，このほか，右顔面神経麻痺，高口蓋がみられた．症例2は，両側網脈絡膜欠損，動脈管開存症，低身長，停留睾丸，小陰茎があり，このほか，左腎低形成，翼状頸，両眼隔離がみられ，頭部 CT にて小脳虫部低形成が疑われた．2例に共通して難聴があり，high resolution CT を行ったところ，中耳あるいは内耳の形成異常が認められた．また，症例1の facial palsy は電気生理学的には末梢側顔面神経障害を認め，顔面神経管を含んだ内耳の形成異常が関与していると思われた．CHARGE Association に伴う中枢神経系奇形としては，無嗅脳症・頭頂後頭葉の低形成・小脳虫部低形成が報告されている．小脳虫部低形成は，今まで3例の報告があり比較的合併しやすい中枢神経系奇形と思われた．

III-33. Development Medical Care and Social Adaptation of Patients with Apert Syndrome: Mitsushiro KIDA (Dept. Pediatr., Teikyo Univ. Sch. Med., Tokyo)

Questionnaire was collected on development medical care and social adaptation of eight patients with Apert syndrome, *i.e.*, 26-year-old man, 11- and 7-year-old girls, 6-, 5-, 2-, 1.5-year-old and 7-month-old girl infants. Among them, one patient died of meningitis at the age of five. The patients' ages were obtained in five cases and the average ages of fathers

and mothers were 34.6 and 30.4 years respectively. An operation for hands was performed in all the patients aged over 2 years and the times of the first operation distributed from 1 year and 1 month to 1 year and 6 month of age. Operation for early cranial fusion was performed in four patients between soon after and 4 months after birth and in one patient at the age of 4. Four patients had an operation for the harelip. It is notworthy that exudative tympanitis was found due to deafness in four patients between 4 years of age and the first grade of elementary school. A 26-year-old man graduated from a high school for the handicapped and worked at the prefectural cooperative workshop for 4 years and now he is working at the distribution center of a supermarket. As to school education, one patient (26-year-old) changed from an ordinary school to a school for the handicapped. The other three patients are attending ordinary school and all of them are satisfied with their school lives. Recently, all who desire are attending preschool. Since Apert syndrome which is not rare, is a typical autosomal dominant hereditary disease, an information about development, medical care and social adaptation of patients with Apert syndrome is very important to the people conducting medical care of the patients.

III-34. A Report on Osaka Birth Defects Monitoring Program, High Risk Factors and Groups: **Noriyuki SUEHARA,[1] Kei-ichi KURACHI,[2] Tosiaki OOURA,[3] Takashi TANIMURA,[4] Jun-ichi FURUYAMA,[5] Osamu TANIZAWA,[1] Makoto IMAGAWA,[6] Sadao TERAMURA,[6] Masao FUKUI,[7] Takashi TAKEMURA,[2] Akira HAYASHI,[2] Akira SASAKI,[2] Tosio FUJINO[8] and Sachio OGITA[9]** ([1]Dept. Obstet. Gynec., Osaka Univ., Osaka; [2]Osaka Med. Cent. Res. Inst. Matern. Child Health, Osaka; [3]Osaka Municipal Rehab. Cent. Disabled, Osaka; [4]Dept. Anat., Kinki Univ., Sayama-cho; [5]Dept. Genet., Hyogo Med. Coll., Nishinomiya; [6]Osaka Med. Assoc., Osaka; [7]Osaka Soc. Obstet. Gynec., Osaka; [8]Child. Med. Cent. Osaka City, Osaka; [9]Osaka Municipal Matern. Infant Cent., Osaka)

The Osaka Birth Defects Monitoring Program was started in December 1981, as a population-based monitoring system for Osaka Prefecture. Twenty-two selected marker malformations and other major malformations were monitored for all live births and stillbirths (over 24 gestational weeks and/or 500 g) within 7 days after birth. In the first 37 months, 181,080 records were collected from obstetric hospitals and clinics in Osaka Prefecture, corresponding about 60% of the births in that period in Osaka Prefecture. The birth prevalence for all malformations in all births was 1.11%; that for the marker malformations was 0.81%. The birth prevalence of total malformations in all babies with low birth weight was 4.09%; that in all premature births was 3.68%. That in small-for-gestational age (SFD) babies among premature births was 14.9%. The birth prevalence of total malformations in neonates whose mother suffered from diabetes mellitus was 4.65%.

III-35. A Family Study of Alpha 1-Antitrypsin Deficiency (PiMnichinan) and Liver Damage: **Haruki NAKAMURA, Toshihiro MARUYAMA, Kazunori TSUDA** (2nd Dept. Intern. Med., Miyazaki Med. Coll., Miyazaki) **and Nariaki MIYAMOTO** (Miyazaki Univ., Miyazaki)

Hereditary α_1-antitrypsin (AAT) deficiency is very rare disorder in Japanese. We studied a family of AAT deficiency (PiMnichinan) and mainly examined their liver functions. The serum AAT concentrations of 2 homozygotes were almost 10% of normal. The proband, 49-year-old female, had almost normal liver function test and normal liver aspect on laparoscopy. Histologically, there were minimal infiltrations of mononuclear cells in portal area and many fat deposits in hepatocytes, but no fibrotic changes were observed. Another homozygote who was 44-year-old sister of the proband exhibited marked hepatomegaly and following data. Serum albumin 3.8 g/dl, γ-gl 1.7 g/dl, GOT 41, GPT 26, γGTP 73, ALP 22K.A., ICG(R15) 8%, HBsAg (−), HBcAb (−). On laparoscopy her liver had irregular surface and dull edge. Liver biopsy specimen showed enlarged portal area and piecemeal necrosis with marked fibrosis and infiltration of mononuclear cells. She was diagnosed as early stage of liver cirrhosis. In their liver biopsy specimen, PAS-positive diastase-resistant globules ranged from 1 to 4 μ in size were observed in the periportal hepatocytes. Eight relatives who were heterozygous subjects of AAT deficiency had normal liver function test. In mechanism of liver damage in AAT deficiency is unknown. It has been postulated that the inhibition of AAT is necessary to limit liver damage caused by the release of digestive enzymes during phagocytosis by Kupffer cells.

III-36. Hypergonadotropic Hypogonadism with Idiopathic Dilated Cardiomyopathy: **Y. TAKAHASHI, K. KIKKAWA, T. DOI, K. NARAHARA, Y. WAKITA, S. KIMURA and H. KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama)

Malouf *et al.* (1985) reported two sister cases with primary hypergonadotropic hypogonadism, congestive cardiomyopathy and other somatic abnormalities. We have recently encountered a female patient similarly affected. The patient, who had been treated for epilepsy, were seen at 18 years of age because of cardiac asthma. There were no families with consanguinity, heart disease and hypogonadism. On physical examination, she had eunuchoid habitus, arachnodactylia, bilateral ptosis, prominent nasal bone and lack of secondary sex characters. The basal levels of LH and FSH in serum were markedly raised, and LH-RH loading test showed excessive and delayed resonses. The karyotype was 46,XX. She died at the age of 19 years. Findings on autopsy included idiopathic dilated cardiomyopathy, ovarian dysgenesis, hypoplasia of uterus, fallopian duct and external genitalia, and no degenerated elastic fibers of the aorta. Idiopathic dilated cardiomyopathy is

seldom associated with ovarian dysgenesis. The phenotypic concordance between our case and the case reported by Malouf *et al.* suggested that such condition may constitute a clinically recognizable syndrome. The specific clinical features are hypergonadotropic hypogonadism, idiopathic dilated cardiomyopathy and characteristic face. Although the present case was sporadic, this condition seemed to be inherited in an autosomal recessive fashion, as indicated by Malouf *et al.* Finally, the disease has to be established as a new form of autosomal recessive XX, gonadal dysgenesis.

III-37. ダウン症候群の皮膚紋理分析 (II)—10 指の紋型出現頻度の性差について—：
浅香昭雄・山田一朗（東大・医・保健），**松井一郎**（国立小児病院医療研究センター・小児生態）．**Analysis of Dermatoglyphics of Down Syndrome (II):**
Akio ASAKA, Kazuaki YAMADA (Sch. Health Sci., Univ. Tokyo, Tokyo) and Ichiro MATSUI (Dept. Child Ecol., Natl. Child. Med. Res. Cent., Tokyo)

ダウン症候群 251 名（男子 140 名，女子 111 名，核型はいずれも 21 trisomy），および健常学童 1,008 名（男子 514 名，女子 494 名）を対象とし，10 指の紋理の出現頻度の性差について検討した．紋理は，弓状紋 (A)，尺側蹄状紋 (U)，撓側蹄状紋 (R)，渦状紋 (W) の 4 分類とした．ダウン症候群では，左手第 5 指，第 4 指，右手第 5 指に出現する紋理の種類に男女間の有意差が認められた．また健常者群では，右手第 1 指，第 2 指，第 4 指，第 5 指において，同じく男女間の有意差が認められた．いずれの場合も，U の出現頻度が女子において高く，W の頻度が男子において高かった．指紋紋理強度 (pattern intensity: PI) の平均値を求めて性差をみると，健常者群では 0.57，ダウン症候群では 0.93 であった．次に，ダウン症候群の男女，健常者群の男女の計 4 群について，数量化II類による重判別分析を行った．抽出された主な二つの因子軸により構成される平面上に，それぞれの因子得点に従って各ケースをプロットしてみた．各群の重心間距離を求めると，健常者群 2.03，ダウン症候群では 3.10 であった．いずれもダウン症候群では，性差がより開く傾向にあることを示すものであった．

III-38. ダウン症候群の皮膚紋理分析 (III)—10 指の紋理型の発生学的類似性の検討—：
山田一朗・浅香昭雄（東大・医・保健），**松井一郎**（国立小児病院医療研究センター・小児生態）．**Analysis of Dermatoglyphics of Down Syndrome (III):**
Kazuaki YAMADA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo) and Ichiro MATSUI (Dept. Child Ecol., Natl. Child. Med. Res. Cent., Tokyo)

10 指の紋理形成の相互関連性について，カテゴリカルデータの解析法のひとつである数量化 III 類を用いて検討した．対象および指紋型の分類は前報と同じである．健常者群においては，抽出された第 1 因子軸にそって各指の W, R, U がかなり接近してプロットされたのに対し，各指の A だけは他の紋理とは独立して，平面上の Y=X の直線上にほぼそった位置にプロットされた．ダウン症候群では，抽出された第 1 因子軸にそって各指の W が，また第 2 因子軸にそって左右第 4, 5 指の R がプロットされた．また，この 2 本の軸により構成される平面上の原点付近に各指の U が，また

原点から第 3 象限寄りの位置に各指の A がプロットされた．抽出された因子軸の意味付けは現段階ではまだ困難であり，今後の実証的な研究成果を待たなければならない．しかし，健常者群とダウン症候群とで 10 指の紋理形成の相互関連性にかなり異なったパターンが認められたことは，それぞれの皮膚紋理の発生に関して異なった機序の存在を示唆したものとして興味深い．

III-39. A Methodological Approach to the Experimental Study of Dermatoglyphics Using the Rat: Michio OKAJIMA and Yaeko ASAI (Dept. Forens. Med., Tokyo Med. Dent. Univ., Tokyo)

Recently, we have found in the rat (*Rattus Norvegicus*) that the dermo-epidermal junction presents dermatoglyphic configurations on the volar pads and digital apices (*Am. J. Phys. Anthrop.* **67**, 81, 1985). For the inspection, the hand and foot were first fixed in 10% formalin and then the epidermis was removed by incubating in 1 N potassium hydroxide solution for 48 hr at 30°C. The dermis exposed by this treatment was stained with 0.05% toluidine blue solution, placed in a dish of water, and inspected under a stereoscopic microscope. As the elevated portion of the dermis is stained in a violet tone, the ridged structure is clearly displayed. We have examined more than 700 rats from closed colonies and inbred strains. The ridges are usually arranged in parallel and in right angle to the longitudinal axis of the pad. On some pads, however, ridge arrangements are characteristic. Particularly, the III interdigital pad of the palm presents various types of patterns such as whorls, loops, arches, triradii, cusps and transitional configurations. Though the anatomical structure is somewhat different from that of primates, it is suggested that the rat, a conventional laboratory animal, is available for the developmental and genetic study of dermatoglyphics as an experimental model.

III-40. 成人病のふたご研究 (I)—血中ブドウ糖レベル，インスリン分泌能に関する検討—：門脇 孝・春日雅人・金沢康徳・高久史麿（東大・医・3内），**赤沼安夫**（朝日生命・糖尿病研），**山田一朗・浮田徹嗣・林 瑾瑩・大沼美喜子・浅香昭雄**（東大・医・保健）．**Twin Study on Adult Diseases (I): Analysis of Blood Glucose Level and Insulin Secreting Capacity:** Takashi KADOWAKI, Masato KASUGA, Yasunori KANAZAWA, Fumimaro TAKAKU (3rd Dept. Intern. Med., Fac. Med., Univ. Tokyo, Tokyo), Yasuo AKANUMA (Inst. Diabetes Care Res., Asahi Life Foundation, Tokyo), Kazuaki YAMADA, Tetsuji UKITA, Chin-Yin LIN, Mikiko OHNUMA and Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo)

東大附属中学校の卒業生を対象とし，成人病を中心とした双生児研究に着手した．昭和 60 年 4 月，調査への依頼状と簡単な質問紙票を同封し，住所の判明している 862 名の双生児に対して発送した．質問紙票は，対象者の性別，年齢，身長，体重，職業，既往歴，家族歴，糖尿病歴，および嗜

好品の摂取状況を問う項目により構成されている．同年 7 月現在の回答数は 335 通である．なお，転居先不明のため返送されたものが 142 通あった．双生児の双方から回答のあったものは 242 通 (121 組) であった．このうち同年 11 月初旬までに，成人病検診の一環としてブドウ糖負荷試験 (GTT) およびインスリン分泌能検査を実施できた MZ 30 組について検討した．なおインスリン分泌能は，糖尿病の予知指標として昨今注目されているものである．グルコース負荷前，負荷後 30, 60, 90, 120, 180 分後の計 6 回にわたり測定した．各計測時点における血中グルコース値の級内相関係数は，順番に 0.52, 0.61, 0.73, 0.58, 0.49, 0.42 であった．インスリン分泌能の級内相関係数は同様に 0.55, 0.49, 0.29, 0.28, 0.09, 0.32 であった．臨床的に有用とされるインスリン分泌指数（ブドウ糖負荷後 30 分間におけるインスリンの上昇値を同時間のグルコースの上昇値で割ったもの）の級内相関係数は，0.64 と高い値を示した．血中グルコース濃度に対応したインスリン分泌能は，遺伝的支配を強く受けていることが推測された．

III-41. 成人病のふたご研究 (II)—身長，体重，既往最大体重についての検討—：浮田徹嗣・山田一朗・林　瑾澄・大沼美喜子・浅香昭雄 (東大・医・保健)，**門脇　孝・春日雅人・金沢康徳・高久史麿** (東大・医・3 内)，**赤沼安夫** (朝日生命・糖尿病研)．**Twin Study on Adult Diseases (II)—Analysis of Height, Body Weight and Maximal Body Weight in the Past—:** Tetsuji UKITA, Kazuaki YAMADA, Chin-Yin LIN, Mikiko OHNUMA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo), Takashi KADOWAKI, Masato KASUGA, Yasunori KANAZAWA, Fumimaro TAKAKU (3rd Dept. Intern. Med., Fac. Med., Univ. Tokyo, Tokyo) and Yasuo AKANUMA (Inst. Diabetes Care Res., Asahi Life Foundation, Tokyo)

第 I 報と同じ対象者について，調査時点における身長，体重および妊娠時を除く既往最大体重の双生児間の分析を行った．身長における級内相関係数は，一卵性双生児 (MZ) 0.96 (N=103)，二卵性双生児 (DZ) 0.61(N=9) であった．体重では，MZ 0.90 (N=102), DZ 0.76 (N=9) であった．既往最大体重においては MZ 0.90 (N=94), DZ 0.76(N=8) となり，いずれも一卵性双生児のほうが二卵性双生児よりも高い相関を示した．出生時身長・体重の情報を検索し，同様に分析してみると，身長・体重ともに一卵性双生児のほうが相関係数は高い値を示した点は同じであるが，本調査時点よりは低い値であった．次に，第 1 子と第 2 子の差について検討した．身長，体重，既往最大体重ともに，ほぼ 30 歳台を境として，第 2 子のほうが大きな値を取るペアが増える傾向にあった．生活史における環境要因のより詳細な分析が必要である．

III-42. 成人病のふたご研究 (III)—飲酒・喫煙行動，食生活習慣についての分析—：山田一朗・浮田徹嗣・林　瑾澄・大沼美喜子・浅香昭雄 (東大・医・保健)，**門脇　孝・春日雅人・金沢康徳・高久史麿** (東大・医・3 内)，**赤沼安夫** (朝日生命・糖尿病研)．**Twin Study on Adult Diseases (III)—Analysis of Drinking, Smoking Behavior and Dietary Habit—:** Kazuaki YAMADA, Tetsuji UKITA, Chin-Yin LIN, Mikiko OHNUMA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo),

Takashi KADOWAKI, Masato KASUGA, Yasunori KANAZAWA, Fumimaro TAKAKU (3rd Dept. Intern. Med., Fac. Med., Univ. Tokyo, Tokyo) and Yasuo AKANUMA (Inst. Diabetes Care Res., Asahi Life Foundatinon, Tokyo)

分析の対象者は，第Ⅰ，Ⅱ報と同じである．飲酒頻度，喫煙頻度については，それぞれ，毎日飲む（喫う）：3点，時々飲む（喫う）：2点，全く飲まない（喫わない）：1点の3段階で回答させた．また1日の喫煙本数をあわせて質問した．飲酒頻度について，双生児間で得点が一致した組の割合は，MZでは78.1% (82/105；3点8組，2点56組，1点18組)，DZでは66.7% (6/9；3点1組，2点4組，1点1組)であった．またスピアマンの順位相関係数を求めたところ，MZで0.68，DZで0.63の値が得られた．同様に，喫煙頻度の一致率はMZ 81.0% (85/105；3点14組，2点3組，1点68組)，DZ 77.8% (7/9；3点3組，1点4組)，順位相関係数はMZ 0.57，DZ 0.75であった．とくにMZの不一致例のなかに，双生児のひとりが3点，他のひとりが1点という組み合わせが13組存在したことが注目された．喫煙本数の級内相関係数は，MZ 0.64，DZ 0.56であった．次に，米飯類，野菜類など16の食品群について，その摂食頻度を質問し，その回答を主成分分析にかけた．「おかず中心」か，「主食中心」かという摂食パターンと関連する第1因子軸と，「洋食中心」か「和食中心」と関連する第2因子軸が抽出された．この二つの因子軸に対する因子得点を求め，その級内相関係数を計算した．第1因子得点は男性独身者（−0.45)，既婚者（0.20）であった．また第2因子得点では，独身者（0.88)，既婚者（0.18）であった．女性の場合は，いずれも正の値であり，独身か既婚かによる変動は男性の場合に比べて小さかった（第1因子得点 0.23→0.38，第2因子得点 0.67→0.48)．食習慣における遺伝要因の存在とともに，それがとくに男性においては，環境要因によって大きく変化することが示唆された．

III-43. 自然排卵による四つ子の卵性診断：塩野　寛・藤原正貴・田畑典子・安積順一（札幌医大・法医)，**阿部正紀**（岩見沢市立病院・小児)．**Tertial Zygosity Diagnosis Due to Spontaneous Ovulation:** H. SHIONO, M. FUJIWARA, N. TABATA, J.J. AZUMI (Dept. Legal Med., Sapporo Med. Coll., Sapporo) and M. ABE (Dept. Pediatr., Iwamizawa City Hosp., Iwamizawa)

四つ子の出産率は，今泉と井上によると，0.94×10^{-6} と報告されている．すなわち1,063,829回の出産に1回の割合で四つ子が生まれることになる．今回われわれは，排卵誘発剤を使用することなく，自然排卵による妊娠で，全員無事に生まれた四つ子の卵性診断を行う機会があり，興味ある結果を得たので報告する．1．症例：母親に早・流産の既往歴はなく，昭和53に男児，55年に女児を分娩している．四つ子出産時の母親年齢は32歳，父親は27歳である．昭和58年4月10日午後3時28分から47分に人工破水で，2,150 g，1,700 g，1,600 g，2,550 g の男児四つ子が全児無事に生まれた．2．検査成績：赤血球型・血清型，赤血球酵素型，HLA型は第1児から第3児はすべて一致していたが，第4児はMN, Rh, Kidd, Gc, AcP, HLAにおいて他の児と異なっていた．したがって第1児，2児，3児が一卵性三つ子，第4児は他の卵による二卵性四つ子と考えられる．

III-44. Twin Study on Alcohol Dependence: Kazuaki YAMADA and Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo)

A twin study was carried out to analyze genetic and environmental factors related to the causes of alcohol dependence. First of all, a nationwide hospital survey was made in order to look for twin cases with alcohol dependence. We could find 15 probands, out of which only 5 pairs, 4 male and 1 female ones, were available for further investigations, including interviews, zygosity diagnosis and so on. The subjects were proved monozygotic through the examination of several kinds of genetic markers. The diagnosis of alcohol dependence was made by DSM-III, and 3 of them were judged discordant. After clinical investigations of their life histories and alcohol-related problems, those who showed intimate relationship with them, such as co-twin, spouse, parents and/or superior officers, were considered to be influential factors on the discordance. A follow-up study on these cases is now in progress.

III-45. Heteromorphisms of Ag-Stained Nucleolar Organizer Regions in Human D and G Chromosomes. II. Heteromorphisms of Ag-NORs in 3 Pairs of Monozygotic Twins: Yoshiaki KODAMA and Akio A. AWA (RERF, Hiroshima)

It is known that the nucleolar organizer regions (NORs) are located on the short arm of D and G chromosomes of man. We reported at the previous Annual Meeting of this Society that the Ag-stained chromosomes did not occur randomly, and that the inter-chromosomal distribution of the Ag-stained NORs (Ag-NORs) showed a consistent pattern for each individual. In the present study, three monozygotic twin pairs were studied for the variation of their NORs. Following whole blood culture, chromosome slides were stained in combination with silver staining and G-banding methods. In each individual, 50 metaphases were analyzed for the number of Ag-positive NORs, irrespective of the size of silver deposits. The approximate size of the Ag-NORs was measured in the following two approaches. The size of the Ag-NORs was scored visually on a graded scale of 0–3 as reported previously. A total of 10 metaphases were analyzed for each case. Secondly, the quantitative measurement of the area of Ag-NORs was performed using a modular system for semiautomatic quantitative evaluation of images (Nikon, COSMOZONE 98, System 1). Enlarged photographic prints by 25,000× magnification from 5 representative metaphases were selected for this quantitative measurement. The results showed that the mean and the modal number of the Ag-positive NORs showed a good concordance between monozygotic twin pairs. Inter-chromosomal differences in the size of silver deposits were observed, but their distribution pattern showed a close association with each of twin pairs. Similar results were obtained from both approaches.

III-46. Four Cases of Fanconi Anemia: Seiji KINOSHITA, Koh NIINOMI, Keiichiro YOSHIOKA (Dept. Pediatr., Osaka Natl. Hosp., Osaka), **Mitsuhiko NANBU, Yasushi HAYAKAWA** (Dept. Pediatr., Tsukaguchi Hosp., Amagasaki), **Shigeki TERADA, Akira YOSHIOKA** (Dept. Pediatr., Nara Med. Univ., Kashihara), **Takahiko SUKENAGA** (Dept. Radiol., Hyogo Coll. Med., Nishinomiya), **Tomoko HASHIMOTO, Atsuko OGAWA, Kiyoshi NAKAMURA and Jun-ichi FURUYAMA** (Dept. Genet., Hyogo Coll. Med., Nishinomiya)

Four cases of Fanconi anemia were reported on clinical findings and the results of chromosomal examination. Pigmentation was found in all cases. Three cases were born as small infants for date. Finger abnormalities, retentio testis, small stature or growth retardation were found in two cases each out of four. Major malformations such as atresia ani or defect of corpus callosum were observed in two patients. Anemia was treated with oxymetholone and two cases responded transiently, but finally required blood transfusions. Remaining two cases have been well controlled with oxymetholone for several months. Chromosomal aberrations were found in all cases more than ten times greater than normal. In two cases, chromosomal aberrations increased after treated with diepoxybutane or mitomycin C. A patient with higher incidence of chromosomal aberrations seemed to develop anemia at younger age. The incidence of chromosomal aberrations of bone marrow cell was as high as that of peripheral blood lymphocyte in one case examined. A sister who does not develop anemia with high chromosomal aberration rate (possible Fanconi anemia) was under study whether anemia would develop or not.

III-47. SV40 DNA トランスフェクションによるブルーム症候群由来線維芽細胞の株化: 吉田独弘（北大・理・染研），**栗原孝行・井上雅雄**（金沢医大・共同研），**大和田幸嗣・中野芳朗・山本義弘・角永武夫**（阪大・微研），**橋本知子・古山順一**（兵庫医大）. **Transformation of Human Fibroblasts from Bloom Syndrome by pSV40-DNA Transfection:** Michihiro C. YOSHIDA,[1] Takayuki KURIHARA,[2] Masao INOUE,[2] Kouji OWADA,[3] Yoshiro NAKANO,[3] Yoshihiro YAMAMOTO,[4] Takeo KAKUNAGA,[3] Tomoko HASHIMOTO,[4] and Jun-ichi FURUYAMA[4] ([1]Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo; [2]Kanazawa Med. Univ., Ishikawa; [3]Res. Inst. Microbiol. Dis., Osaka Univ., Osaka; [4]Hyogo Coll. Med., Hyogo)

ブルーム症候群 (BS) 由来線維芽細胞 (BS1KA, BS2KA) の株化トランスフォーメションを，SV40 DNA トランスフェクションにより行った．SV40 全ゲノムを組み込ませた pBR322 プラスミドより DNA を得て，リン酸カルシウム沈殿法により BS 細胞にトランスフェクした．DNA 導入後，3 日目あるいは 7 日目に継代培養を行ったのちに 5 週目でのコロニーまたはフォーカス形成をみた．7 日ごとの継代培養を繰り返したものがコロニー形成能が高く，また，コロニー形成にはキャリア DNA を必ずしも必要としなかった．DNA 導入をしなかった BS 細胞は数週間の継代培養でクラ

イシスに入ったのに対して，pSV40 DNA 導入により増殖性を獲得した細胞はそれよりさらに継代培養が可能であり，BS1KA および BS2KA ともに高発の SCE を保持していた．

III-48. Ethylnitrosourea Hypersensitivity of Bloom's Syndrome Fibroblasts: Takayuki KURIHARA and Masao INOUE (Cent. Res. Lab., Kanazawa Med. Univ., Kanazawa)

We attempted to elucidate the ethylnitrosourea (ENU) hypersensitivity of Bloom's syndrome (BS) fibroblasts by investigating the repair-time-dependent reduction kinetics of 2 BS (BS1KA, BS2KA) and 1 normal (N7KA) strains in ENU-induced SCEs. BS cells were 5-fold more sensitive to ENU in inactivation of colony forming ability than normal cells. The induction rate of immediate SCEs by ENU in BS cells was about 80 times higher than that in normal cells. In normal cells, the reduction of ENU-induced SCEs as a function of repair time showed a biphasic curve of the first rapid (1–6 hr) and the second plateau (6–48 hr) components. At the plateau, the level of ENU-induced SCEs was 133% of untreated control (7.3 SCEs/cell). However, in BS cells, the ENU-induced SCEs decreased slowly with the repair time and the level reached 133% of untreated control (51.4 SCEs/cell) at 48 hr. These results indicate that BS cells are lacking in the rapid excision which reduce rapidly ENU-induced SCEs in normal cells.

III-49. Sister Chromatid Exchanges: Sex Difference in Base-line and Mitomycin C-Induced SCE Frequencies: Mimako NAKANO and Akio A. AWA (Dept. Genet., RERF, Hiroshima)

Comparison of the SCE frequencies between the two sexes was made from data obtained with normal young individuals aged 13–14 at examination born to A-bomb survivors (F1), since the chance of being exposed to potential SCE-inducible environmental mutagens such as smoking is lower for young persons than for aged adults. Each blood sample from 14 female and 10 male donors was divided into two cultures: one treated with mitomycin C (MMC) at a concentration of 10 ng/ml, and the other not (control). Attempts were made to employ both SCD and G-banding stains on the same metaphases from 2 female and 2 male volunteers. Mean base-line SCE frequency was 8.52 ± 0.84 for females, and 7.87 ± 1.00 for males, respectively. Another result from both SCD and G-banding stains showed a mean value of 7.75 for females and 7.28 for males, respectively. Values in females were higher on both occasions than those in males. These observations have confirmed our previous data derived from three independent studies. In MMC-treated culture, a mean SCE frequency was 28.75 ± 2.10 for females and 28.41 ± 2.17 for males, respectively. SCEs seemed to occur in proportion to the chromosome lengths in both control and MMC-

treated cultures. SCE frequencies in autosomes were almost the same between the two sexes. In regard to the sex chromosomes, however, SCE frequency of two X chromosomes in females was significantly higher than that of X and Y chromosomes in males. The difference seems to be attributable to the chromosome lengths. These results suggested that the base-line SCE frequency in females was slightly higher than that in males but not statistically significant, and that the sex difference in SCE frequency might be due to the difference in the lengths of the sex chromosomes.

III-50. Cytogenetic Studies on Childhood Non-Hodgkin's Lymphoma: Toshiro NISHIDA, Akira YAMAGISHI, Keiko WAKUI, Masaaki YAMADA (Div. Lab., Saitama Child. Med. Cent., Saitama), **Yasuhide HAYASHI, Yuji HABU, Ryoji HANADA, Keiko YAMAMOTO** (Div. Hematol. Oncol., SCMC, Saitama) **and Takashi ABE** (Dept. Pediatr., Keio Univ., Tokyo)

Using G, Q banding techniques, chromosomal analyses were made on peripheral blood, bone marrow, lymph node or pleural effusion from 11 patients with non-Hodgkin's lymphoma. These included 5 cases of lymphoblastic lymphoma (LL), a case of diffuse lymphoma (DL), and 5 cases of Burkitt's lymphoma (BL). Immunologic studies indicated that all of the 5 cases with LLs were T cell origin, one case with DL was non-T non-B cell origin, 4 out of 5 BLs were B cell origin, and the rest was pre B cell origin. All specimens had clonal abnormalities. Among T cell lymphomas a partial loss of a long arm of No. 6 was found in 3 cases, the breakpoint showing q21 in 2 cases, q23 in one case, and abnormality of a short arm of No. 9 was found in one case. A translocation 8;14 was found in 5 cases with BL, and the breakpoints showed q24 and q32, respectively. Tetraploid chromosome abnormality including 4q+ was found in one case with DL and the karyotype showed 92,XXXX,−1,−4,+2der(4)t(1;4)(q21;q35). A t(8;14) was possibly confined to BL, and a 6q− and 9p− may be characteristic for LL.

III-51. Sequential Chromosome Abnormalities in B-Cell Chronic Lymphocytic Leukemia: A Study of 13 Cases: Kazuo OHTAKI, Tin HAN and Avery A. SANDBERG (Dept. Genet. Endocrinol., Roswell Park Memorial Inst., USA)

Some investigators have reported for the specific chromosome abnormality in lymphocytes stimulated by polyclonal B-cell activators in B-cell chronic lymphocytic leukemia (B-CLL) to be trisomy 12. Other abnormal clones such as trisomy 3, 18 and 14q+, 14q− have also been observed. The chromosomal constitutions of stimulated lymphocytes in 13 patients with B-cell chronic lymphocytic leukemia (B-CLL) were sequentially examined using polyclonal B-cell activators (PBA), *i.e.*, Epstein-Barr virus (EBV), lipopolysaccharide W from

E. coli (LPS), pokeweed mitogen (PWM) and protein A from *Staphylococus aureus* (PA). Of the eleven patients (44 samplings) with abnormal clones, two patients had only trisomy 12, 6 patients had trisomy 12 plus other clonal abnormalities such as +8, +9, +16, +18, 6q−, 15q+ and t(4;15), and the remaining 3 cases had various clonal abnormalities other than trisomy 12, such as trisomy 3, 8, 20, 21, insertion of 7 and 12. These findings suggest that even though trisomy 12 may be a common abnormality in B-CLL, various other abnormal clones may also be present *in vivo* for relatively long periods of time.

III-52. Immunogenetics of Adult T-Cell Leukemia (II): Hisamitsu UNO, Kiyohide KAWANO, Hitoshi MATSUOKA, Nobuyoshi TACHIBANA and Kazunori TSUDA (2nd Dept. Med., Miyazaki Med. Coll., Miyazaki)

In order to elucidate immunogenetic factors involved in the pathogenesis of adult T-cell leukemia (ATL), HLA was typed for 51 patients with ATL, which were composed of 32 acute type, 9 chronic type, 7 smoldering type and 2 unclassified. Also typed were 30 patients with T-cell malignant lymphoma (T-ML), of which 60% were positive for anti-HTLV-1 antibodies, and 63 healthy carriers positive for the antibodies. ATL showed the association with HLA-Bw62 ($P_c < 0.01$), especially with acute type ($P_c < 1 \times 10^{-4}$). HLA-A26 and Cw3 which are in linkage disequilibrium with HLA-Bw62 were also increased. Since T-ML positive for the antibodies were indistinguishable from lymphoma type of ATL, patients with ATL combined with T-ML positive for the antibodies were analyzed. The combined patients showed the same trend in frequencies of HLA antigens with patients with ATL. HLA-B7-DR1 haplotype was not observed in acute ATL. This was significant compared with healthy carriers ($P_c < 0.003$). Natural killer (NK) cell activity to K562 and Molt4 cell lines did not differ between ATL, healthy carriers and healthy control group. NK activity to HTLV-1 virus producing T-cell lines, MT-2 and HUT102, was not observed in three groups. A serum from an patient with acute ATL enhanced NK activity to MT-2 cell line. The enhancement of NK activity was not observed when other cell lines were used for target cells. Analysis of the factor(s) of the serum is now under investigation.

III-53. Chromosomal Abnormalities in a Case of Erythroleukemia before and after DCMP Therapy: t(1;21), 7q−, dic, Ring, Minutes *etc.*: Mariko UEHARA, Mitsushiro KIDA (Dept. Pediatr., Teikyo Univ., Tokyo), **Tomiko RYU and Takeshi ABE** (1st Dept. Intern. Med., Teikyo Univ., Tokyo)

A 64-year-old man was diagnosed as erythroleukemia in January, 1985. Abnormal chromosomes observed in bone marrow cells included del(1)(p22), del(3)(p14p25), two t(1;21)

(p22;p11), three del(7)(q22), dic, small ring(sr), large ring(lr) including double ring of short arm of chromosome 1, small minute (m_1), double minutes (m_2), −5, −20, PCD (premature centromere division), endoreduplication, 4n, *etc.* The 1p− and 3p− chromosnmes were observed in all the karyotypically abnormal cells. Other abnormal chromosomes were variable in number per cell and in percentage of cells. All abnormal karyotypes showed any one or two of dic, r and m. However, dic chromosomes did not coexist with r and m in the same cell. Normal karyotypes were observed in 2 of 16 cells (1-day culture), and in 2 of 14 cells (4-day culture) only after DCMP therapy (daunomycin, cytosine arabinoside, 6-mercaptoprine, prednizolone). The variation in chromosome number distribution increased after therapy and decreased *in vitro*. The modal number of chromosome was constantly 48. The percentages of each cells with two t(1;21), three 7q−, dic, or m_2 decreased after therapy, whereas the cells with one t(1;21), 7q−, 2r, 3r or m_1 increased after therapy. This suggests that the former cells are sensitive and the latter cells are resistant to the therapy. Furthermore, the incidence of the former cells increased and that of the latter decreased during three-days culture before the therapy, indicating that the former had a higher cell growth advantage in culture than the latter. However, after the therapy a reverse relationship was observed.

III-54. Lung Tumor and Ensuing AML(M2)t(8;21) Developed in a 47,XY,+mar Male:

Masako MINAMIHISAMATSU (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba), **Juana S. GREGORIO** (Philippine Atomic Energy Com., Quezon City), **Yasusuke ONOZAWA** (Dept. Med., Tokyo Metropol. Komagome Hosp., Tokyo) **and Takaaki ISHIHARA** (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba)

A patient with lung tumor and consecutive AML(M2)t(8;21)(q22;q22) was found to have a constitutional supernumerary chromosome. The patient was a 52 year old male who developed large cell carcinoma on his right lung and underwent pulmonary resection on the anterior and posterior lobes in May, 1981. He was readmitted to the hospital because of respiratory distress due to an infectious disease of the lung in February, 1985. AML(M2) was detected by the blood examination, and the presence of translocation t(8;21) and the supernumerary chromosome was found by the chromosome examination. Since the supernumerary chromosome was present not only in leukemic cells but also in cells from skin, CFU-F and non-leukemic marrow and in T-lymphocytes, it was certainly a constitutional one. The supernumerary chromosome was metacentric, consisting of C-positive chromatin, and had satellites and Ag-positive NOR on both arms. It is possible that the chromosome was an isochromosome of a short arm of one of the five pairs of chromosomes having satellites, namely, Nos. 13, 14, 15, 21 and 22. The patient was phenotypically normal and mentally superior. In the AML cells an interstitial deletion (q11→q22) on the long arm

of No. 9 was also observed in addition to the t(8;21) translocation and the marker. The increased incidence of malignant disorders such as leukemias has been known in Down's syndrome and others having numerical chromosome abnormalities. The presence of the supernumerary chromosome in the case reported here might have some relation to the development of its AML and lung tumor.

III-55. 第 2 番染色体長腕部分欠失例での遺伝子座位の検討：山中　昴・宮崎　清（愛知コロニー・中央病院），**大石英恒・小笠原信明**（愛知コロニー・発達障害研），**小林正紀**（名古屋市大・医・小児）．**A Case of 2q Terminal Deletion and Regional Mapping of Chromosome 2:** T. YAMANAKA, K. MIYAZAKI (Cent. Hosp., Aichi Pref. Colony, Kasugai), H. OISHI, N. OGASAWARA (Inst. Develop. Res., Aichi Pref. Colony, Kasugai) and M. KOBAYASHI (Dept. Pediatr., Nagoya City Univ., Nagoya)

症例は 5 歳の女児．両親は血族結婚でなく，出生時父親 27 歳，母親 26 歳であった．妊娠 2～3 カ月時に切迫流産による出血があった．在胎 39 週，2,030 g で出生．小頭症，粗な頭髪，前額部突出，眼裂狭小，眼裂斜下，斜視，耳介の異常，前向きの鼻孔，歯列不整，小下顎症，短頸，細長い指などの症状があり，染色体検査で第 2 番染色体の異常を指摘された．1 歳過ぎに G, R バンド分染法を行い，46,XX,del(2)(q35) と診断した．両親の染色体には異常を認めなかった．体重 6.0 kg (−5.5SD)，身長 78 cm (−7.45SD) と著明な発育障害があり，おすわりは不能で，発語もなく，重度の精神運動発達の遅れがみられた．2q32-qter に遺伝子座位があるとされる isocitrate dehydrogenase, soluble (IDH1) と ribulose 5-phosphate 3-epimerase (RPE) の酵素活性を患児の赤血球で測定したところ，両酵素ともコントロールとくらべてほぼ半分の活性値を示した．これは IDH1, RPE の遺伝子座位が本症例の欠失部分にあるための遺伝子量効果によると思われた．以上の結果より，IDH1, RPE の遺伝子座位は 2q35-qter に存在すると考えられた．

III-56. Interferon-γ Production by Human Peripheral Lymphocytes in Retinitis Pigmentosa: Atsuko MORIMOTO, Masahiko KOMORI, Keiko FUJIKI, Atsushi KANAI, Akira NAKAJIMA (Dept. Ophthalmol., Juntendo Univ., Tokyo), **Katsuro NATORI and Shudo YAMAZAKI** (Cent. Virus Diagnostic Lab., Natl. Inst. Health, Tokyo)

Some immunological aberration has frequently been reported in some population of retinitis pigmentosa (RP). Hooks, J.J. *et al.* (1983) described the depressed production of interferon-γ (IFN-γ) in RP patients. In this study ten Japanese patients with RP (including six autosomal recessive, one autosomal dominant, two sporadic, and a patient who has a cousin with RP) were investigated to examine whether the production of Con-A induced IFN-γ from peripheral lymphocytes were depressed or not by using the viral CPE inhibition assay. The titers of IFN at 24 and 48 hr after incubation with Con-A was not significantly

lowered, compared with that of ten healthy controls although the titers at 72 hr were rather higher than that of controls. This tendency seen in patients was not dependent on the mode of inheritance. Our results showed no significant defect in IFN-γ production in RP patients contrary to the previously reported data. Because of the relatively higher titers at the last incubation time in RP patients there still remains the possibility that RP patients have some factors which have influence on the ability of IFN-γ production by the peripheral lymphocytes.

III-57. Localization of the EGF Receptor (EGFR)/c-*erb*B Protooncogene to the p14–p12 Region of Chromosome 7: Nobuyoshi SHIMIZU, John HUNTS (Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo), **G. T. MERLINO, J. WANG-PENG, Y.-H. XU and I. PASTAN** (Lab. Mol. Biol., NCI, Bethesda, MD.)

The human EGF receptor gene (EGFR) has been previously localized to the p13–q22 region of chromosome 7 by analyzing the expression of human specific EGF receptors in two series of human-mouse somatic cell hybrids (*Cytogenet. Cell Genet.* **35**: 9, 1983). These cell hybrids were produced by fusing mouse A9 cells that are deficient in EGFR with the human diploid fibroblast lines GM1356, 46,XX,t(1;7)(q34;p13), and GM2068, 46,XX, t(6;7)(q27;q22), both of which possess EGF receptors. We analyzed these hybrids using Southern hybridization with probes, pE7 and pEB1, for the EGFR and v-*erb*B genes, respectively. The DNA restriction patterns produced by *Eco*RI and *Hin*dIII agree well with the p13–q22 region for these gene sequences. That is, presence of the sequence homologous to EGFR and v-*erb*B genes in the former series of cell hybrids was correlated with the retention of the human translocation chromosome containing the 7p13–qter region, and in the latter series of cell hybrids it was correlated with the retention of the human translocation chromosome containing the 7pter–q22 region. Furthermore, *in situ* hybridization to chromosomes from normal human lymphocytes showed that the EGFR probe hybridized to the p14–p12 region of chromosome 7. These results support the idea that EGF receptor gene (EGFR) constitutes a c-*erb*B protooncogene and these genes are located on the p14–p12 region of chromosome 7.

III-58. Education of Medical Genetics: How to Educate Psychological Aspects: Hide-aki CHIYO, Hiroko KAWASHIMA and Masio KITATANI (Dept. Clin. Genet., Inst. Hum. Genet., Kanazawa Med. Univ., Ishikawa)

The importance of psychological aspects of genetic counseling has been emphasized among some of the medical geneticists. When we try to offer any genetic services to the patients with genetic disorder, psychological techniques such as counseling technique and/or

crisis intervention are, undoubtedly, very important especially in telling the diagnosis and in assisting or encouraging the clients to take preferable decisions in their behaviors. The authors have been tried to educate the students on medical course the psychological aspects in the curriculum of human genetics. As to the teaching methods, small group discussion using audio-visual system and role playing method showed good results. Simulation test in essay form which was demonstrated here was thought to be one of the practical methods for evaluation.