日本人類遺伝学会第 28 回大会一般講演要旨

Abstracts of General Contribution, the 28th Annual Meeting of the Japan Society of Human Genetics

一 般 講 演 General Contribution

A1. 家族性大腸ポリポーシス患者における染色体の高精度バンド解析，とくに No. 2 染色体についての検討：池内達郎・外村 晶（東医歯大・難研），宇都宮譲二（兵庫医大・二外），岩間毅夫（東医歯大・二外）．**High-Resolution Chromosome Analysis in Patients with Familial Polyposis Coli, with Special Reference to the No. 2 Chromosome:** T. IKEUCHI, A. TONOMURA (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ.), J. UTSUNOMIYA (2nd Dept. Surg., Hyogo Med. Univ., Nishinomiya), and T. IWAMA (2nd Dept. Surg., Tokyo Med. Dent. Univ., Tokyo)

家族性大腸ポリポーシス (FPC) は常染色体性優性遺伝病とされているが，他の大部分の優性遺伝病と同様に，その遺伝的素因の実体は不明である．最近，Gardner ら (1982) が，FPC と Gardner 症候群の患者には No. 2 染色体の長腕近位領域 (q14.3～q21.3) の部分欠失が検査例すべてに認められたことを報告して注目された．そこでわれわれは，互いに家系の異なる FPC 患者 5 例について染色体高精度解析を行った．末梢血培養に臭化エチジウム前処理法を適用し 550～850 バンド・レベルの G-分染像を示す細胞を各例につき少なくとも 10 個以上選び，とくに No. 2 相同染色体間における分染パターンの照合を試みた．その結果，いずれの症例においても，2q21 の淡染バンドの大きさに変化はみられず，さらに q14.3 と q22 の濃染バンドとの間に検出される q21.2 の小さな濃染バンドが常に定位置に存在することを確認した．すなわち，No. 2 長腕部には識別しうる部分欠失は認められず，前記の知見を追認することはできなかった．他の優性遺伝病である網膜芽細胞腫や無虹彩-ウイルムス腫瘍の患者における近年の染色体知見から，高発癌性の遺伝的素因として染色体の微小部欠失を想定することは十分に妥当と思われる．しかし，これを染色体レベルで立証するには，十分に精度の高い多くの分染像を観察することが肝要で，できればバンドの計測値に頼ることなく，1 個のサブバンドの有無あるいはその位置的なずれの検索といった定性的な解析を行うことが重要であろうと思われる．

A2. Analysis of Heteromorphism of Chromosome No. 2 in Patients with Hereditary Adenomatosis of the Colon and Rectum: Toshihiko KASUKAWA (1st Dept. Surg., Yamagata Univ. Sch. Med.), **Toshiaki WATANABE and Akira ENDO** (Dept. Hyg. Prev. Med., Yamagata Univ. Sch. Med., Yamagata)

Gardner *et al.* (1982) reported the presence of a heteromorphism of chromosome No. 2 homologues (they have tentatively identified it as a deletion of 2q(14.3→21.3) in a homo-

logue) in cultured lymphocytes and fibroblasts from patients with hereditary adenomatosis of the colon and rectum (ACR) and their children at risk for multiple adenomas. This report prompted us to ascertain whether this heteromorphism is present in the ACR patients by measurement analysis of prometaphase and early metaphase chromosomes. The magnitude of the difference (δ) in relative lengths of 2q(cen→21.3) to the whole chromosome between the homologues (longer minus shorter) was compared among three ACR patients and three control donors. Mann-Whitney U-tests on the δ's among all combinations between individual ACR patients and individual control donors did not reveal any significant difference. This analysis showed that variations of relative lengths between a pair of homologues and variations of the δ's among homologues are considerable both in the ACR patients and in the control donors. In order to establish the presence of chromosome No. 2 heteromorphism as a cytogenetic marker of ACR, further studies may be necessary. At present, it can be stated that not all patients with ACR possess heteromorphism of chromosome No. 2.

A3. A Case of Ring Chromosome 3: Mashio KITATANI, Junya YASUDA, Chen Cheng CHEN, Fukumi IDA, Hiroaki TAKAHASHI and Shoichiro SHIKE (Dept. Pediatr., Kanazawa Med. Univ., Ishikawa)

The patient, a girl, was the second child of healthy nonconsanguineous parents; the maternal age was 34 years and paternal age was 41 years at the time of the proband's birth. An older sister was a healthy 9 years girl. Pregnancy was uneventful. Delivery was normal at 39 weeks of gestation; birth weight was 1,900 g, length 44.0 cm and head circumference 28.0 cm. She was slow to feed initially. She held her head at 3 months, smiled at 4 months and sat at 9 months. She was admitted to our hospital for investigation at 10 months. She then weighed 5,680 g (<3rd centile), measured 67.5 cm (=3rd centile), had a head circumference of 36.0 cm and developmental age 9 months. The routine blood tests, urinalysis, creatinine, T_3, T_4, TSH, urine amino acids and X-rays all gave results within normal range. The following malformations were observed; microcephaly, narrow face, epicanthal folds, flat nasal bridge, down-turned corners of the mouth, wide nipples, incurved fifth fingers and proximally implanted thumb. Her dermatoglyphs from thumb to fifth finger were W, W, W, W, A on the left and W, W, W, W, U on the right hand. She had intermediate axial triradius. The karyotypes of the parents were normal. The patient had 46,XX,r(3)(p26q29) karyotype in 77.4% of peripheral lymphocytes. The remainder cells had dic r (3) or rod-shaped r (3) or interlocked r (3) in aneuploid.

A4. 比較的稀な4番染色体異常症の2例：Distal 4q monosomy と distal 4q trisomy：
北島晴夫（栃木県身障医療福祉センター・小児）

症例1：9か月男児．妊娠分娩歴に異常なく，在胎37週2,350 gにて出生．仮死(−)．父29歳，母29歳．家族歴に異常はない．新生児期より大泉門の膨隆があり，2か月時，某大学病院で冠状縫合早期閉鎖による短頭症と診断され頭蓋局所切除術を受けた．その後，上気道感染を繰り返し，神奈川県立厚木病院受診．狭頭症を伴う短頭，小顎症，耳介変形，翼状頸，足趾重畳，猿線，短四肢などの変質徴候がみられ，核型は46,XY,del(4),q27であった．両親は，正常核型であった．4q monosomyの本邦初例である．既報4例 (q31) とは切断点を異にし，臨床像にも明らかな相違があり，Townesの提唱する 4q− syndrome と同一疾患とは考えにくい．

症例2：7歳女児．妊娠2か月時，切迫流産の既往があり，第1度仮死にて出生．在胎40週3,400 g．母29歳，父31歳．家族歴に異常はない．生下時より体重増加不良あり，某医で先天性心疾患 (VSDとPDA) を指摘され，3歳時動脈管切断術を受けた．術後，右片麻痺を生じた．6歳時，栃木県身障医療福祉センターに入所．小頭，低身長，前額部突出，内眼角贅皮，蒙古様眼裂，歯列不整などを認め，核型は46,XX,21p+(4q26 or 27→qter)であった．両親は正常核型で，distal 4q trisomyの珍しい *de novo* type 症例である．

A5. A Case of 7q Partial Monosomy: Madoka ARISAKA, Toshiaki SUGAWARA, Hanako TADA, Hiroshi TAKAHASHI (Dept. Pediatr., Juntendo Univ., Sch. Med., Tokyo) **and Tamiko SHINOHARA** (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent., Tokyo)

The patient was a four month old female who was the first child of unrelated and healthy parents. She was born after 35 week-gestational period. Birth weight was 1,040 g. Immediately after birth, she was admitted to Dept. of Pediatrics, Juntendo University, because of abdominal distention and frequent vomittings. She had a peculiar facial appearance and the following abnormalities were observed: Microcephaly, bulbous nasal tip, low-set and large dysplastic ears, micrognathia, abnormally clenched hands and a single palmer crease in the left hand. She failed to thrive. At the age of 4 months, her weight was 2,850 g and her length 46 cm. Her physical and mental developments were retarded. Psychometry showed DQ 30. Hypertonicity of the muscles in all four extremities was observed. CT-scan showed slight dilatation of the ventricle. Chromosome preparations with the trypsin-Giemsa banding technique were carried out from peripheral blood lymphocytes and skin fibroblasts. Chromosome analysis revealed a structural abnormality, 46,XX,7q−. The karyotype of the patient was tentatively identified as 46,XX,del(7)(q32), but the chromosome analysis using high-resolution band was not yet performed. Hageman factor (XII) activity of the patient revealed 36% decrease, but the activity of her mother showed 30% decrease. These findings suggest that the locus of Hageman factor is not located on the deleted segment of No. 7.

A6. A Case Report of Systemic Abnormalities with Bilateral Congenital Corneal Staphylomas: Hideo TAKI, Mayumi YAMAMOTO, Hiroshi TAMAI, Seiichi SHIMADA, Masahisa FUNATO (Dept. Pediatr., Yodogawa Christian Hosp., Osaka) **and Fusako FUJIMOTO** (Dept. Ophthalmol., Y.C.H.)

Congenital corneal (anterior) staphyloma is a rare developmental abnormality characterized by a totally opaque and ectatic cornea that bulges forward through the palpebral fissure. The condition may be bilateral or unilateral; in unilateral cases the unaffected eye has another anomaly. Our case is bilateral and associated with systemic abnormalities, such a case has not been reported previously, to the best of our knowledge. The patient, born on May 3, 1983, was the first child of healthy unrelated parents (maternal age 23 years, paternal age 25 years, nationality-Korea). The pregnancy was uncomplicated and she was delivered by vacuum extraction. The mother denied usage of drugs, exposure to X-rays or toxins during pregnancy. She was noted at birth bilateral dense corneal opacities and other systemic abnormalities, and so she was transferred to our hospital. There was no history of neurologic disease, ophthalmologic disease, nor congenital malformations. She had the following congenital abnormalities: (1) the midline occipital scalp defect, (2) coarse hair, (3) congenital corneal staphyloma, (4) congenital localized lymphangioma (left anterior chest), (5) funnel chest, (6) snapping fingers, (7) hypogenesis of toes, (8) hypogenesis of nails. TORCH-titer; not elevated. IgM; 2 mg/dl. The cytogenetic analysis revealed a karyotype, 46,XX,9qh+. The mother had the same karyotype. The father had normal karyotype.

A7. A Female Case with 46,XX,inv dup(12)(q24.2-qter) Associated with a Peculiar Face, Broad Thumbs and Big Toes: Atsushi IESHIMA, Taeko YORITA (Div. Child Neurol., Tottori Univ., Yonago) **and Shigeru OHTA** (Dept. Pediatr., Matsue Red Cross Hosp., Matsue)

Ten cases with partial 12q trisomy have been reported. We report an additional case with pure distal 12q trisomy. The proposita was the second child of a healthy 27-year-old mother and father. She was born at full term, birth weight 3,083 g, after normal pregnancy and delivery. At the age of 5 months, she was referred to our clinic because of failure to thrive and odd looking. The main clinical features included short stature (−3.9 SD), psychomotor retardation, cardiac defect (VSD), prominent forehead, hypertelorism, low-nasal bridge, down-turned mouth, short neck, wide-set nipples, sacral dimple, broad thumbs and first toes, and hypoplastic nails. Dermatoglyphics showed ridge dissociation of bilateral fifth fingers, decreased a-b ridge count (63), and third interdigital pattern. Radiological study showed broad distal phalanx of thumbs and first toes, disproportionally advanced bone age of carpal and tarsal bones. Cytogenetic study used

with lymphocyte revealed the karyotype of 46,XX,inv dup(12)(q24.2-qter) by G- and R-banded techniques. The parental karyotypes were normal. Previous reports were 12q trisomy deriving from translocation or duplication 12q mosaicism. Present case is the first report of pure distal 12q trisomy. Previous cases as well as our case have many common, characteristic features, so distal 12q trisomy is a clinically recognizable syndrome.

A8. Clinical and Anatomopathological Findings of a Case with a Ring Chromosome 22: Tadao ARINAMI and Susumu NAKAJIMA (Ibaraki Pref. Colony Hosp., Ibaraki) **and Ikuko KONDO** (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

We report clinical and autopsy findings of a patient with a ring 22 chromosome who died at 27 years of age. He was born at term weighing 2,175 g, after an uncomplicated pregnancy and delivery to a 30-year-old mother and a 33 year-old father. His brother and sister were normal. Clinical features, at 20 years of age, were the following: height 158 cm, weight 50 kg, head circumference 52 cm, profound mental retardation, hypertonicity, cutis verticis gyrata, flat occiput, full eyebrows, long eyelashes, hypertelorism, high nasal bridge, large nose, large ears, gynecomastia, and kyphosis. Dermatoglyphics revealed proximal loops on both thenar areas. The karyotype was identified to be 46,XY,r(22) by G-banding technique. He died after having had episodic symptoms such as apathy, insomnia, anorexia, vomiting, masked-like facial expression, tremor of the upper extremities, instability of gait, and convulsions. Postmortem examination revealed (1) a brain with a weight of 1,090 g, (2) heterotropic islands of nerve cells in the cerebral white matter, (3) an anomalous multilocular arachnoid cyst at the left medullo-cerebellar angle with marked compression atrophy of the surrounding brain tissue, resulting in supratentorial internal hydrocephalus, and (4) multiple, bean-sized or smaller-sized nodules of meningeal cell hyperplasia attached to the dura mater cerebri and to the arachnoid cyst wall.

A9. Turner 症候群 45,X の姉妹例：藤田弘子（大阪市大・児童保健），**谷川洋子**（兵庫県立塚口病院・検査），**松本秀雄**（大阪医大・法医）．**Recurrence of the 45,X Turner's Syndrome in Sisters:** Hiroko FUJITA (Child Health, Osaka City Univ., Osaka), Yoko TANIGAWA (Tsukaguchi Hosp. Lab., Hiyogo), and Hideo MATSUMOTO (Dept. Legal Med., Osaka Med. Sch., Osaka)

典型的 Turner 症候群の臨床像をもつ長女（11 歳）と三女（3 歳）に 45,X 核型を検出した．父は 27, 35，母は 24, 32 歳時の出産である．両親は，特記すべき既往歴や流死産歴はない．父は 19～20 歳時（昭和 33～34 年）胸部レントゲン検診車に数か月従事し，学童の撮影介助を行って頻回の照射を受けている．本人は，無資格で防御衣なしに作業をしたと陳述した．Xg^a 血液型は父(+)，母(+)，長女未検，三女(−)であり，妹は父の X を消失したものと推定される．母の染色体は正常女性核型であり，

モザイクを示唆される所見は得られなかった．父の核型も大多数は正常男性であるが，177 観察細胞中少なくとも 5 核板に異常細胞を見いだした．1) 47, Bq−, +mar（着糸点指数が B 群より小さい A 群大の異常染色体），2) 46, Bq+, −C, −18, +r(?), 3) 46,r(?), 4) chromatid gap のある 2 細胞である．父は X 線暴露後 20 年経ているが，照射によると思われる染色体障害所見が存在していた．45,X 姉妹例は，Dunlap ら (1972) の報告がみられるにすぎない．本症例では，父が青年期の精祖細胞増殖期に受けた X 線により break が生じ，構造異常で不安定な性染色体を消失した精子の受精したものではないかと推定する．

A10. Variation of Centromeric and Karyotypic Mosaic in a tdic(13;18)(p11;p11) Case. Effect of MMC, EB and BrdU: Mariko UEHARA, Mitsushiro KIDA and Koichi YOSHIMURA (Dept. Pediatr., Teikyo Univ., Tokyo)

The clinical features of the male infant were colpocephaly, short stature, prominent forehead, low set ears, hypertelorism, broad based nose, micrognathia, clinodactyly of fifth finger, and simian crease. Karyotype of the patient were analysed using lymphocytes from the patient between four and thirteen months of age. Complex mosaic of karyotypes and centromeric types were observed with variation. (1) 45,XY,t(13;18)(p11;p11), 69–93%, (2) 46,XY,t,+13p−, 0–4%, (3) 46,XY,t,+18p−, 1–4%, (4) 46,XY,13p−18p−, 0–14%, (5) 46,XY,t+t, 0–3%: (A) dic(primary constriction at both), 16–53%, (B) pseudo dic(primary at chromosome 13, 0–12%; at chr. 18, 0–4%), (C) two types of monocentric, 33–84%. Dic and pseudo dic decreased with age. Induction effect on fission of dic-[karyotype(4)] were observed by treatment with MMC 0.01 μg/ml for 24 hr, and EB 10 μg/ml for 2 hr before harvest. On the contrary reduction effect were observed by adding BrdU 2 μg/ml at start of culture. Other karyotypic anomalies observed were r(13), i(13) *etc.* in 0–1%, and 4n in 0–4%. Anaphase bridges and breakage were observed in 6% and 2% out of total anaphase in culture without colcemide. Probably in this patient's early cleavage stage, partially dic changed to pseudo dic and monocentric, then other complex karyotypes arised by fission of dic and non-disjunction following anaphase bridge and breakage. Karyotype with fission of dic-(4) was lost in one or two cell cycle *in vitro* and *in vivo*.

A11. Diploid/Tetraploid Mixoploid in a Long Survival Case: Chiharu TAKADA, Hide-aki CHIYO, Hiromi SAKAMOTO, Tomoko HASHIMOTO, Miyako YABUKI, Jun-ichi FURUYAWA (Dept. Genet., Hyogo Coll. Med., Nishinomiya), **Norimitsu OHTSUKA** (Dept. Cent. Lab., Hyogo Coll. Med., Nishinomiya), **Susumu TSUJI** (Dept. Pediatr., Hanwa Hosp., Osaka) **and Hideo MATSUMOTO** (Dept. Legal Med., Osaka Med. Coll. Takatsuki)

A case of diploid/tetraploid mosaicism was demonstrated in a 3 year-old girl. Main clinical features are severe mental retardation and motor impairment, accompanied by

multiple minor anomalies. Two other cases of the same chromosomal condition have been reported. However, each case presents different clinical features, thus it is somewhat difficult to define the diploid/tetraploid condition as a clinical entity, *i.e.*, a syndrome. The clinical differences observed in the cases reported so far, are probably due to the different mechanisms involved in the origin of this condition, which would result in the various proportions and/or locations of the abnormal cells.

A12. A Medical Study on the Etiology of Mental Retardation. Hereditary Diseases, Known Syndromes of Non-Chromosomal Etiology and Chromosomal Aberrations: **T. IKEDA, C. MIYAGI, T. HOKAMA, K. HIRAYAMA** (Dept. Pediatr., Ryukyus Univ., Okinawa), **S. OHDO, H. MADOKORO** (Dept. Pediatr., Miyazaki Med. Coll., Miyazaki), **K. NARITOMI, K. SAMESHIMA, T. TERAWAKI** (Dept. Pediatr., Kagoshima Univ., Kagoshima) **and H. TANAKA** (Natl. Sanat. Minami-Kyushu Hosp., Kagoshima)

Although mental retardation (MR) exhibits a relatively higher prevalence which is estimated at about 3% of the population, about 40% are due to unknown etiology according to the previous studies. We reported the frequency based on periodic classification, and percentage of chromosomal aberrations, known syndromes of non-chromosomal etiology and hereditary diseases. Subjects were 866 children who were diagnosed MR or suspected MR and visited one of Departments of Pediatrics, Ryukyus University, Miyazaki Medical College, Kagoshima University, and National Sanatorium Minami-Kyushu Hospital. Results. 1) The frequency based on periodic classification: Prenatal was most frequent, 75.6%; perinatal, 5.5%; postnatal, 0.1%; undecided age at onset, 18.7%. 2) The frequency based on etiologic classification: (a) The most frequent causes were chromosomal abnormalities, 30.0%. (b) MR due to known syndromes of non-chromosomal etiology was 5.9%. (c) MR due to hereditary diseases was 5.9%. 3) Clinical diagnosis was unknown in 27.8%.

A13. Five Patients with Chromosome Aberrations Found in a Mental Hospital: **Bunnoshin ISHIKAWA** (Utsunomiya Mental Hosp., Tochigi) **and Akio ASAKA** (Dept. Mental Health, Univ. Tokyo, Tokyo)

Five patients with chromosome aberrations were found among 61 ones who were selected from 974 inpatients because of their having "Degenerationszeichen." Diagnoses of these 61 patients were mental retardation (n=24), epilepsy (n=9), schizophrenia (n=13), alcoholism (n=6), drug dependence (n=4), psychopathy (n=2) and other mental disorders (=3). Out of 5 patients with chromosome aberrations 4 were found from mental retardation and remaining one from epilepsy. Their karyotypes were 47,XYY, 47,XXY, 46,

XY,t(3p+;8q−), 46,XY,t(3q+;6q−), 46,XY,inv(9p+q−). It was suggested that signs of dermatoglyphics were most effective and gave clinicians useful quick informations among "Degenerationszeichen" or minor anomalies.

A14. 慢性リンパ性白血病の染色体異常：貞森直樹（長崎大・医・原研・内科），**A.A. SANDBERG**（米国ローズウェルパーク記念研究所）. **Chromosomal Aberrations of Chronic Lymphocytic Leukemia:** N. SADAMORI (Dept. Int. Med., Atomic Disease Inst., Nagasaki Univ., Nagasaki) and A.A. SANDBERG (Dept. Genet. Endocrinol., Roswell Park Memorial Inst., New York)

米国ローズウェルパーク記念研究所における B 細胞型慢性リンパ性白血病 62 症例について，Epstein-Barr vivus (EBV), lipopolysaccharide (LPS), pokeweed mitogen (PWM), Cowan I などの polyclonal B-cell mitogen (PBM) を使用し染色体検査を実施した．sister chromatid differentiation 法を用いた PBM による刺激後の細胞周期については，48 時間目にはほとんどの分裂細胞は M_1 期にあり，72 時間目には約 50% の分裂細胞は M_2 期に，また 96 時間目には 60% 以上の分裂細胞は M_3 期にあることが判明した．各種 PBM による染色体異常を有する細胞頻度は，PWM や Cowan I に比べ，EBV や LPS によって刺激された材料に最も高率に見いだされた．62 症例の対象のうち，51 症例 (82%) に分裂像が得られ，20 症例に染色体異常がクローンとして認められた．これら染色体異常は，11 症例にトリソミー 12，2 症例に 14q+，2 症例にトリソミー 18 が見いだされた．また，M 蛋白が検出された 5 症例すべてにトリソミー 12 が認められた．マーカー染色体は病期の進行した症例に多く，予後の予知的指標になりうることを示唆した．

A15. Two Cases of Congenital Acute Lymphocytic Leukemia with the t(4;11) Chromosome: Tamiko SHINOHARA (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent., Tokyo), **Atsushi SHIBUYA and Toshio YOSHIKAWA** (Dept. Pediatr., Saitama Med. Coll., Saitama)

Two cases of congenital acute lymphocytic leukemia with a translocation (4q−;11q+) are reported. Case 1: The patient was a two day old female who was admitted to Dept. of Pediatrics, Saitama Medical College, because of petechiae in her whole body, hemangioma-like tumor in the left lower extremity, abdominal distention and hepatosplenomegaly at birth. On admission, WBC was 7,200 with 98% of lymphoblasts. Bone marrow aspiration revealed hypercellularity with a remarkable increase of lymphoblasts. The diagnosis of ALL of Common ALL type was made, using lymphocyte surface marker analyses, *i.e.* E rosette(−), EA rosette(−), surface immunoglobulin(SIg) (−), Ia-like antigen (80%), Common ALL antigen (60%). Furthermore, using IgM immunofluorescence method, the diagnosis of pre B ALL with cytoplasmic IgM(+) was made. Chromosome constitution of the leukemic cell was 46,XX,t(4;11)(q12;q23). Case 2: The patient was a two month old male who was admitted to Kawaguchi Saisei-kai Hospital, because of

abdominal distention, anemia and hepatosplenomegaly. On admission, WBC was 266,000 with 97.6% of lymphoblasts, and bone marrow aspiration revealed hypercellularity with 77.8% of lymphoblasts. The diagnosis of ALL of Common ALL type was made on the basis of lymphocyte surface marker analysis of the leukemic cell, *i.e.* E rosette(−), SIg(−), Ia-like antigen (60%), Common ALL antigen (80%). Chromosome constitution of the leukemic cell was 46,XY,t(4;11)(q12;q23). In the reference, it was suggested that the acute leukemia associated with t(4;11) represents a proliferation of an early myeloid progenitor cell. One of our cases was pre B ALL with cytoplasmic IgM(+), and the other was ALL of Common ALL type. These findings suggest that the two lymphocytic leukemias might be a proliferation of the bone marrow precursor cells that have the capacity to differentiate towards both myeloid and lymphoid cells.

A16. Cytogenetic Studies on Hemopoietic Precursors in Chronic Myelogenous Leukemia: Yoshiaki SONODA, Taira MAEKAWA, Kazuhiro NISHIDA, Masafumi TANIWAKI, Tatsuo ABE and Tatsuro TAKINO (3rd Dept. Intern. Med., Kyoto Pref. Univ. Med., Kyoto)

Cytogenetic studies of myeloid (CFU-C) and erythroid (CFU-E, BFU-E) precursor cells were performed in 32 patients with Ph^1-positive chronic myelogenous leukemia (CML). In the chronic phase the Ph^1-negative cells were found in CFU-C obtained from 7 out of 18 (38.9%) cases and those incidences were ranging from 1.4% to 60%. The Ph^1-negative cells were also found in 1 out of 7 (14.3%) cases with blastic CML. In the other 8 patients, cytogenetic studies were performed in CFU-E and BFU-E. The Ph^1-negative cells were detected in 4 out of 8 (50%) cases. The present data disclosed that the Ph^1-negative normal committed stem cells persist both in myeloid and erythroid precursors. How ever, the incidences differed from those of the precursors and varied with clinical stages. This study also demonstrated that suppressor/cytotoxic T-cells were uniformly Ph^1-negative. Recently, Martin *et al.* (1980) demonstrated that B-lymphocytes were involved in the malignant clone. Although CML is a clonal disorder of the pluripotent stem cell, more extensive work is required to elucidate whether T-lymphoid cell lineage is involved in original CML clone.

A17. The Origin of Mosaic Down Syndrome: Norio NIIKAWA (Dept. Pediatr., Hokkaido Univ., Sapporo) **and Tadashi KAJII** (Dept. Pediatr., Yamaguchi Univ., Ube)

Five children, two girls and three boys, with 46/47,+21 mosaic Down syndrome were studied for the mechanism of origin of mosaics, using Q- and R-banding heteromorphisms

as markers. Three mosaics started as a trisomic zygote followed by the loss of a chromosome 21 at an early mitotic division. Of these, one resulted from a maternal first meiotic nondisjunction, another from a paternal first meiotic event, and the third originated from a first meiotic error in either parent. The remaining two subjects could have resulted from either a diploid or trisomic zygote. These findings, together with higher proportion of trisomic cells in skin fibroblasts than in peripheral blood lymphocytes in two patients studied, suggest that the extrachromosome 21 in Mosaic Down syndrome patients predominantly has a meiotic origin. In two, possibly three, of the diploid cell lines in these mosaics, "uniparental" chromosomes 21, namely, both the homologous members were derived from a parent.

A18. The Origin of an Extrachromosome 13 in Patau Syndrome: Satoshi ISHIKIRIYAMA and Norio NIIKAWA (Dept. Pediatr., Hokkaido Univ., Sapporo)

Four patients with a standard trisomy 13 were studied for the origin of an extrachromosome by the use of the sequential QFQ- and RFA-banding methods. One 13-trisomic originated from a nondisjunction at the maternal first meiosis, another from a paternal first meiotic nondisjunction, and the remaining two could have originated either from a maternal second meiotic error or from a nondisjunction involving a maternally derived chromosome 13 at the first cleavage division. These findings, together with those in previous studies gave a conclusion that 75% of the nondisjunction occur in the maternal meiosis, which is comparable to the value for Down syndrome.

A19. The Effects of Scavengers to γ-Ray Induced Chromosome Aberrations in the Lymphocytes with 21 Trisomy. II. Ethanol: Fumiko SAITO,[1] Sho MATSUBARA[2] and Akira TONOMURA[1] ([1]Dept. Cytogenet., [2]Dept. Radiol., Tokyo Med. Dent. Univ., Tokyo)

Last year we compared the frequency of exchange-type aberrations induced by γ-ray irradiation between normal and trisomic cells after treatment with cysteamine which scavenges some free radicals produced by indirect action of irradiation. In the present study, we used ethanol as a scavenger. Lymphocytes from Down's syndrome and normal control were treated with ethanol at various concentrations (0, 0.01, 0.1, 0.5 and 1 M) and the cells were irradiated by γ-ray (300 R, 98 R/min), washed twice and then incubated for 48 hr at 37°C in RPMI-1640 medium supplemented with 20 per cent fetal calf serum. The chromosome preparations were made according to the standard procedure. The frequencies of dics.+rings were 0.42, 0.42, 0.38, 0.25 and 0.23 per cell in the control and 0.63, 0.49, 0.45, 0.31 and 0.23 per cell in the trisomy at various concentrations of ethanol. The

results showed that the reduction in the frequency of aberration was more remarkable in the trisomic cells than in the normal cells. The frequency of the terminal deletions decreased with the concentration of ethanol in both the normal and trisomic cells. These results suggest that ethanol has more protective effect against irradiation in the trisomic cells than in the normal cells on the production of chromosome aberrations.

A20. Relationship between the Parental Origin of the X Chromosomes, Embryonic Cell Lineages and X-Inactivation Pattern in Mouse Triploid Embryos: Sumiyo ENDO, Nobuo TAKAGI and Motomichi SASAKI (Chromosome Res. Unit. Fac. Sci., Hokkaido Univ., Sapporo)

Using BrdU-labeling and acridine organge staining method, we studied X chromosome replication pattern in 73 digynous and six diandrous mouse triploid embryos obtained from mating between superovulated chromosomally normal A/He females and males carrying Cattanach's X chromosome. The autosomal insertion that substantially increases the length of the Cattanach X chromosome facilitated to distinguish X chromosomes from both parents. Every triploid embryo was mosaic for two cell lines, one with one synchronously replicating, presumably genetically active, X chromosome and the other with two such X chromosomes. The distribution of these two cell types was not uniform within each embryo. In both digynous and diandrous triploids, the embryonic ectoderm tended to have one synchronously replicating X chromosome which was either maternal or paternal in origin. In the trophectoderm and primitive endoderm lineages, however, two X chromosomes tended to replicate synchronously in digynous triploids, whereas only one X chromosome tended to replicate synchronously in diandrous triploids. These isocyclic X chromosomes were nearly exclusively maternal in origin. These findings further corroborate the view that X-inactivation is potentially random in the embryonic ectoderm and the paternal X chromosome is preferentially inactivated in other cell lineages.

A21. Replication Patterns of the Inactivated X Chromosome in Mouse Preimplantation Embryos: Osamu SUGAWARA, Nobuo TAKAGI and Motomichi SASAKI (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Based on the BrdU-labelling acridine orange fluorescence technique, we examined X chromosome inactivation in primary tissues of female mouse embryos before implantation. In 3.5-day blastocysts, the allocyclic X was found in 43 of 272 cells. It was early replicating in 39 of 43 cells. That trophectoderm is responsible for the asynchronously replicating X was verified by (1) the failure to find any cell with an asynchronously replicating X chro-

mosome in immunosurgically isolated ICMs, and (2) out of 48 mural trophectoderm cells mechanically isolated from giant blastocysts formed by aggregation of five or six 8-cell embryos 6 had an early replicating X and 1 had a late replicating X chromosome. The situation in the primitive endoderm was studied in 3.5-day ICMs cultured for 24 hr. An early replicating X was found in 5 out of 85 metaphases from ICMs with the primitive endoderm layer, whereas such allocyclic X was never detected in immunosurgerized ICMs. Thus, it may be concluded (1) that X inactivation occurs first in the trophectoderm and subsequently in the primitive endoderum, (2) that X inactivation does not occur simultaneously in all cells of each tissue, (3) that the inactivated X chromosome in these tissues is early replicating, and (4) that cell differentiation slightly precedes X inactivation detected by replication asynchrony.

A22. Maternal Aging and Nondisjunction: A Comparative Study of Two Chromosomal Techniques on the Formation of Univalents in the Mouse: Shigeki SUGAWARA* and Kazuya MIKAMO (Dept. Biol., Asahikawa Med. Coll., Asahikawa, *Present address: Teijin Inst. Bio-med. Res., Tokyo)

The incidence of univalents was compared between slides prepared according to two different chromosomal methods, *i.e.*, Tarkowski's method and ours, in order to clarify whether a univalent pair can be formed artificially at the first meiotic metaphase (MI), using the oocytes obtained from young (2–3 mo) and old (12–15 mo) age groups of both C57BL/6 and dd mice. Despiralized, fuzzy and loosely associated chromatids were seen frequently in the slides prepared according to Tarkowski's method, while such features were seen less frequently in the slides prepared according to our method. The incidence of oocytes with univalents in the slides made by Tarkowski's method was much higher ($p < 0.05$–$p < 0.001$) than in those made by ours in both age and strain groups. Thus, it was confirmed that the so-called univalents could be produced artificially. The artifacts occurred very frequently in oocytes obtained from old females, indicating that bivalents of aged oocytes are predisposed to be dissociated artificially. The incidence of univalents was significantly higher in the chromosomal group with the 5 smallest ones than in the group with 15 medium-large ones, showing that the bivalent association in small chromosomes is fragile and, thus, tends to form artificial univalents.

A23. Selective Elimination of Chromosomally Unbalanced Zygotes at the Preimplantation Stages in the Chinese Hamster: Shin-ichi SONTA (Dept. Genet., Inst. Develop. Res., Aichi Pref. Coll., Kasugai)

The zygotic selection of genome imbalance was investigated in the Chinese hamster by direct chromosome analyses of preimplantation embryos from the cross between chromosomally normal females and males heterozygous for reciprocal translocations [T(2;10)-3Idr, T(1;3)7Idr and T(1;2)9Idr]. The frequency of zygotes karyotyped successfully from the experimental cross (+/+ ♀ × T/+ ♂) was consistent with that of embryos from the control mating (+/+ ♀ × +/+ ♂). The ratio of embryos karyotyped successfully at the second cleavage metaphase, however, decreased in the experimental cross compared with the control mating. On the other hand, embryos with a specific karyotype which was seen at the first cleavage metaphase were no longer seen after the second cleavage metaphase. The common abnormality of these embryos was partial monosomy of chromosome 2. Among day 4 embryos karyotyped successfully, some embryos mainly with deficiency of the chromosome segment had fewer cells as compared with chromosomally balanced embryos. The findings indicate that embryos with such deficiency delay their cleavage by day 4 of gestation. Furthermore, the increase of preimplantation losses of ovulated ova suggests that some of them are eliminated before implantation.

A24. Isolation and Characterization of the cDNA Clones and the Genomic Clones of the HLA Class II Antigen: Kikuo TSUKAMOTO, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Hidetoshi INOKO, Asako ANDŌ** (Dept. Molec. Biol., Keio Univ., Sch. Med.), **Tadaaki HIROSE and Seiichi INAYAMA** (Pharm. Inst., Keio Univ., Sch. Med., Tokyo)

cDNA clones encoding β chains of human major histocompatibility complex class II antigens were isolated from a human cDNA library. This library was made from partially purified poly A^+ RNA from the human lymphoblastoid cell lines AKIBA (HLA-DR2, Dw12, DC1) using the G·C tailing technique. Two sets of 14-base long oligo DNA were synthesized based on the amino acid sequence of the regions conserved between DR2 and DC1 β chains. Five independent cDNA clones were screened from the cDNA library with synthetic oligo DNA as a probe. These class II β clones were analyzed by restriction endonuclease mapping, and could be grouped into three subsets. The first, pDCβ-101, pDCβ-102 and pDCβ-103 represent same mRNA derived from a single class II gene corresponding most likely to the DC subregion. The another one, pDCβ-201 encodes a DC β chain and the rest, pDRβ-101 contains a sequence specific for a DR β chain. By comparison of the restriction map analysis between pDCβ-101 and pDCβ-201, it was revealed

that these two clones have conserved sequence corresponding to β-2 domain and 3′ untranslated region, but have completely different β-1 domain and N-terminal sequences. This result reveales that at least two distinct loci encoding DC β chains are expressed. We also isolated several lambda phage clones of class II β chain genes from human genomic gene library screened with pDCβ-101 as a probe, and we estimate at maximum seventeen class II genes exist in HLA-D region.

A25. 網膜芽細胞腫を伴う No. 13 染色体長腕欠損の一症例：高精度バンド解析による欠損部位の同定とエステラーゼ D 遺伝子座位の検討. **池内達郎**（東医歯大・難研），**西垣逸郎**（愛知コロニー・発達障害研・遺伝），**藤木慶子・桑原洋子・中島 章**（順天堂大・医・眼科）. **Interstital Deletion of Chromosome 13q Associated with Retinoblastoma: Identification of the Deleted Segment by High-resolution Chromosome-banding and Evaluation of Esterase D Gene Locus:** T. IKEUCHI (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), I. NISHIGAKI (Dept. Genet., Inst. Develop. Res., Aichi Prefect. Colony, Aichi), K. FUJIKI, Y. KUWABARA, and A. NAKAJIMA (Dept. Ophthalmol., Sch. Med., Juntendo Univ., Tokyo)

網膜芽細胞腫 (RB) を伴う 13q− 症例の共通な欠失部は q14 で，この部位にはエステラーゼ D (ESD) 遺伝子座位が存在することも知られている．しかし，各症例における欠失領域のより正確な同定と ESD の表現型に基づいた信頼度の高い活性値の検討がさらに必要と思われる．本報告例は，4 歳の女児（本学会第 25 回大会に報告の Shinohara *et al.*, 1980 と同症例）．両側性の RB と軽度の精神発達遅延以外に顕著な先天異常はない．両親（母 33 歳，父 31 歳）および姉（6 歳）は健康．患児の末梢血培養に臭化エチジウム前処理法を適用して，染色体の高精度解析を行った．通常の分染標本では del(13)(q12q14) と認定されたが，550～700 バンド期の分染像を多数観察したところ欠失領域は q12.3→q21.2 であることが確認された．両親の核型は正常であった．一方，Nishigaki *et al.* (*Ann. Hum. Genet.*, **47**: 187, 1983) の方法で患児と両親および姉の ESD を検索し，それぞれの表現型に対応する平均活性値（1,300 名の計測値に基づく）を含めて次の結果を得た．母親：表現型 1 型，活性値 6.18 units/gHb (1 型の平均活性値 5.7)．父親：2-1 型，4.31 (4.1)．姉：1 型，5.97 (5.7)．患児：2 型 (2-0 型)，1.47 (2.5)．すなわち，定性および定量的知見ともに ESD 座位が上記欠失領域内に存在することを示した．なお，患児の ESD 表現型は母親側座位の欠損を反映しているが，このことは異形性 No. 13 染色体の観察からも立証できた．本症例の欠失領域には q14 バンドは完全に含まれており，以上の結果は RB および ESD 座位に関する従来の知見と矛盾しない．

A26. Assignment of Gene Coding for Isocitric Dehydrogenase, Soluble (IDH_1) to 2q33.1→q33.3: **Kouji NARAHARA, Kiyoshi KIKKAWA, Shunsuke KIMURA, Hiroshi KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama), **Ryozo KASAI** (Asahigawa Jidoin Child. Hosp., Okayama), **Shinya NAGAI and Yohei NISHIBAYASHI** (Dept. Pediatr., Matsuyama Red Cross Hosp., Matsuyama)

High resolution banding techniques coupled with gene dose study in a case with a chromosomal duplication or deletion would make it possible to determine more accurately the localization of a certain gene locus on a given chromosome. The gene for isocitric dehydrogenase, soluble (IDH_1) has been assigned to 2q32→qter. In order to pinpoint the site of this gene locus, we studied red cell IDH_1 in 4 unrelated cases with partial trisomy or monosomy for the long arm of chromosome 2 identified by high resolution banding techniques. Case 1, 1 year and 3 month-old male infant, showed typical features of the trisomy 2q syndrome. The karyotype was 46,XY,der(7), t(2;7)(q33.3;q36.3)mat. Case 2, a 4-year-old girl, had speech delay but no distinct malformations. She was monosomic for 2q33.1→q34. Case 3, a 6-year-old girl, showed growth failure, severe psychomotor retardation, microcephaly, low set ears and finger abnormalities. The karyotype was 46,XX,del(2)(q23q24.2). Case 4, a 2-month-old male infant, showed cleft lip and palate, ptosis, hypertelorism, macrostomia, ear malformations, heart murmur and bilateral cryptorchidism. Direct intrachromosomal duplication of 2q11.2→q24.2 was detected at band of 2q11.2. IDH_1 activities were assayed according to the method of Bergmeyer and Bernt (1974) with modifications, and the enzyme was separated using starch gel electrophoresis (Harris and Hopkinson, 1976). The level of red cell IDH_1 activity was 40% of normal in Case 1 and 50% in Case 2, while it was normal in Cases 3 and 4. Since the father of Case 1 also had a reduced value of IDH_1 activity, the lowered level in Case 1 was ascribed to a rare IDH_1 variant. Electrophoretic studies of erythrocytes and lymphocytes could not determine whether the variant was type 1-5 or 1-6, or an actual new kind of variant was involved. The presence of gene dose effects in Case 2 with monosomy for 2q33.1→q34 but the lack of that in Case 1 with trisomy for 2q33.3→qter led us to assign the IDH_1 gene locus to the region 2q33.1→q33.3.

A27. Assignment of a Human Pan-T Cell Surface Antigen Gene to Chromosome 6: **Noboru KUZUMAKI, Tsuneyuki OIKAWA, Seiji YAMAGIWA** (Lab. Genet., Cancer Inst., Hokkaido Univ. Sch. Med., Sapporo) **and Toshiyuki YAMADA** (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Monoclonal antibody 5B3, directed against a cell surface antigen T72 present on human thymus, T lymphoid cells and cultured T lymphoma cell lines (Ishii, Y. *et al.* 1983. *Clin.*

Exp. Immunol. 53: 31), were used to identify the human chromosome that codes for the antigenic determinant. A human adult T cell leukemia cell line MT-1 expressed T72 antigen, and no cross-reactivity was showed with a mouse thymoma BW5147. Eighteen BW5147 × MT-1 hybrid clones were studied simultaneously for antibody binding as detected by indirect immunofluorescence and for human chromosome content by a combined Hoechst/quinacrine mustard staining (Yoshida, M.C. *et al.* 1975. *Proc. Jpn. Acad.* 51: 185). Concordancy with binding of MoAb 5B3 was observed only for human chromosome 6. All other chromosomes were excluded by six or more discordant hybrid clones. Therefore, we assigned the gene for T72 antigen to chromosome 6. This conclusion was also supported by the concordancy of expression of both T72 and HLA antigens; previous work had assigned the gene controlling the expression of the latter antigen to chromosome 6.

A28. Gene Dosage Study for Galactose-1-Phosphate Uridyl Transferase (GALT) in Three Unrelated Cases with 9p Duplication or Deletion and Regional Mapping of the GALT Locus to 9p13.1→p21.2: **Ryozo KASAI** (Asahigawa Jidoin Child. Hosp., Okayama), **Akira HATA, Yozo ICHIBA, Tomotaka MATSUSHITA** (Dept. Pediatr., Natl. Okayama Hosp., Okayama), **Naoki KATAOKA** (Dept. Pediatr., Kawasaki Med. Schl., Kurashiki), **Kouji NARAHARA, Kiyoshi KIKKAWA and Hiroshi KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama)

The gene locus for GALT has recently been assigned to the p21 or p13 band of chromosome 9 (Human Gene Mapping Conference, 1981). We studied the red blood cell GALT activities in 2 cases of 9p trisomy and one case of 9p monosomy, in order to determine the accurate locus for GALT. Case 1, a 2 year and 10 month-old boy, showed typical features of the 9p trisomy syndrome. The karyotype identified by the high-resolution banding technique was 47,XY,+der(9), t(9;21)(p13.1;q21.2)mat. Case 2, a 3-month-old female infant, had some features of the 9p trisomy syndrome, and her karyotype was interpreted as 46,XX,inv dup(9) (pter→p24.3::p21.2→p24.3::p24.3→qter). Case 3, a 6-month-old male infant, showed psychomotor retardation, brachycephaly, hypertelorism, saddle nose, low-set ears and webbed neck, and the karyotype was 46,XY,del(9)(p22p24.1). His phenotype differed from the monosomy 9p syndrome. The red blood cell GALT activities were measured according to the method of Beutler (1975). Case 1 had an elevated level of red blood cell GALT activities, while Cases 2 and 3 had normal activities. The level of red blood cell GALT activities in Case 1 was 1.5 times a mean value of his parents (Case 1 31.3 IU/gHb, father 22.7 IU/gHb and mother 19.9 IU/gHb), indicating triple gene dosage effects in Case 1. These findings strongly suggested that the GALT locus can be assigned to the p13.1→p21.2 band of chromosome 9.

A29. Regional Mapping of Human Chromosome 4: Assignment of the Genes for Albumin, Group-Specific Component and Phosphoglucomutase-2 to 4pter→q25: **Hidetsune OISHI, Itsuro NISHIGAKI** (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai) **and Tsutomu YAMANAKA** (Cent. Hosp., Aichi Pref. Colony, Kasugai)

The structural genes for albumin (ALB) and group-specific component (GC) have been assigned to 4q11→q13 and for phosphoglucomutase-2 (PGM2) to 4p14→q12 (*Human Gene Mapping* 7, 1983). In addition to our previous report (*Jpn. J. Hum. Genet.* 1980), we present another evidence for the regional assignment of these genes which was obtained using two cases with partial trisomy of chromosome 4. The first case, a boy with multiple anomaly was born on Aug. 18, 1982, after 40 weeks of gestation, as the second child of a 28 year-old mother and a 32 year-old father and weighed 2,780 g. The karyotype of the patient was 46,XY,der(15),t(4;15)(q25;p1), while the parents had normal chromosomes. The second case, a boy with multiple anomaly was born on Oct. 4, 1979, after 38 weeks of gestation, as the first child of a 25 year-old mother and a 34 year-old father and weighed 2,920 g. The karyotype of the patient was 46,XY,der(9),t(4;9)(q31;p24)pat. His mother had normal chromosomes. All of these parents had no record of irradiation and were not consanguineous. ALB and GC systems in serum were examined and the electrophoretic separation revealed no abnormal inheritance in phenotypic patterns. Electrophoretic pattern to fhe PGM2 in red cells showed the same phenotype in individuals examined and their relative activities were almost in normal range. Therefore, it was considered that the genes for these markers were still retained in loci of normal composition of the chromosomes.

A30. ヒト造血器腫瘍由来永代樹立細胞株の細胞遺伝学的研究．V. 培養株でみられた特異的染色体変化ならびに *onc*-genes との関連について： **阿部達生・山根洋子・西田一弘・谷脇雅史**（京都府医大・3 内，臨検），**広瀬正雄**（徳島大・小児），**湊啓輔・下山正徳**（国立がんセンター・内科）．**Cytogenetic Studies on Established Lines Derived from Various Hematological Malignancies. V. On the Primary Chromosomal Changes and Their Relevance to the *onc*-Genes Mapped on the Chromosomes:** Tatsuo ABE, Yoko YAMANE, Kazuhiro NISHIDA, Masafumi TANIWAKI (Depts. Med. and Clin. Invest., Kyoto Pref. Univ. Med., Kyoto), Masao HIROSE (Dept. Pediatr., Tokushima Univ., Tokushima), Keisuke MINATO and Masanori SHIMOYAMA (Dept. Med. Natl. Cancer Center, Tokyo)

造血器腫瘍由来樹立株のうち，T cell line 14 株，B cell line 14 株，non-T non-B 4 株，non-lymphoid 7 株の計 39 株について，G-, C- バンドと DAPI などを用いた連続染色法を行い，染色体分析を行った．構造異常に involve された染色体を cell line 別に多い順にあげると，B では，No. 14 全株，

No. 8, 11 株, No. 13, 9 株, No. 1, 8 株, No. 4, 7 株, No. 3, No. 11, 各 6 株であった. T については昨年の本学会で発表したので省略する. non T non B では, No. 5, No. 12, No. 13, 各 3 株, non-lymphoid では, No. 9, No. 17, 各 3 株であった. また各 cell line に共通して多く認められた異常は, T で, 2p および 6q での欠失あるいは転座であった. B では 14q+ が 12 株に認められ, その donor はほとんどが 8q であり, ほかに 13q における転座も多く観察された. non lymphoid cell line は由来も, AML, APL, AMoL, CML-BC などと様々であったため, 共通した異常を把握できなかった. cell line 独自に観察された異常は, T における No. 14 の関連した derivative および No. 16 を含んだ dicentric 染色体, B にみられる 11q の duplication であった. 染色体上に map された *onc*-genes との関連は, c-mos, c-myc の座位のある No. 8, c-myb の No. 6, c-ras^{H} の No. 11, c-ras^{K} の No. 12 に比較的多数の切断点が観察された. 今後の研究に待たれる点が多いが, 樹立株は *onc*-genes の研究に好都合な材料といえる.

A31. Studies on Characterization and Staining Affinities of Acridine and Quinoline Derivatives to Human Chromosomes: Kouichi MAMBA, Mutsuo KITAHAMA (Dept. Legal Med., St. Marianna Univ. Sch. Med., Kawasaki) **and Akira UCHIUMI** (Natl. Chem. Lab. Indust., Tsukuba)

Four kinds of synthesized acridine derivatives, 9-acridyl-4-dimethylaminophenylhydrazone (ADH), 9-acridyl-4-methoxyphenylhydrazone (AMH), 9-acridyl-2-furanylhydrazone (AFH) and 9-acridyl-2-furanacrolylhydrazone (AFAH), and five kinds of synthesized quinoline derivatives, 2-quinolyl-4-dimethylaminophenylhydrazone (QDH), 2-quinolyl-4-methoxyphenylhydrazone (QMH), 2-quinolyl-2-furanylhydrazone (QFH), 2-quinolyl-2-furanacrolylhydrazone (QFAH) and 2-quinolylphenylhydrazone (QH) were examined for their characterizations and staining affinities by fluorescent light microscopy to develop new fluorescent dyes to human chromosomes. In acridine derivatives, the color of fluorescences showed orange yellow, the absorption maximum values were between 440 and 495 nm, and the dissociation constants (pK_a) were between 5.40 and 6.25. In quinoline derivatives, the color of fluorescence showed yellow green, the absorption maximun values are 356 and 416 nm, and the dissociation constants (pK_a) were between 5.40 and 6.25. Among these derivatives, AMH, ADH, AFH, QDH, QMH and QFH showed the distinctive good staining affinity to human chromosomes.

A32. ヒト精子染色体の直接分析法の改良：上口勇次郎・美甘和哉 (旭川医大・生物).
A Methodological Improvement for Direct Analysis of Human Sperm Chromosome: Y. KAMIGUCHI and K. MIKAMO (Dept. Biol. Sci., Asahikawa Med. Coll., Asahikawa)

最近, ゴールデンハムスター卵にヒト精子を受精させ, その染色体を直接分析する方法が開発されたが, 分析の成功率はかなり低い (用いた卵の 5～10%). われわれは従来の方法に以下の諸改良を

加え，より高い成功率（50～60%）を得ることができた．すなわち，1) 受精能獲得に用いる血清アルブミンと各人の精子の間の親和性にはしばしば大きな個体差が認められるので，各調査例ごとにまず6種類のアルブミンを用いて予備テストを行い，最も成績の良いものだけを実験に用いる，2) 精子をイオノフォア A_{23187} で処理し，短時間で確実に受精能を獲得させる，3) これらの処理によって高率に受精能を獲得した精子を用い，低い精子濃度で短時間受精を行う，4) 受精卵を TCM-199 で培養する，5) 従来用いられてきたコルセミドではヒト精核とハムスター卵核の合核阻止や紡錘糸形成阻止が難しいので，代わりにビンブラスチンとポドフィロトキシンの混合液を用いる，6) われわれの開発した，成功率・信頼度の高い卵子染色体標本作製法を用いる，の6点である．この改良法を用いて，現在まで精子由来の核板178例を染色体分析した．その結果，異数体の出現頻度（4例，2%）は従来の報告（5～7%）より低く，一方，構造異常の出現頻度（19例，11%）は従来の報告（2～4%）よりかなり高かった．われわれの調査例のうちには構造異常の出現頻度がとくに高いものも含まれていたので，さらに例数をふやして検討する必要がある．

A33. An Isolation of Human Chromosomes by Cell Sorter: Keiko MOMOI, Masaharu ISOBE, Yoshinori KUMURA, Zen-ichi OGITA (Dept. Biochem. Pathol., Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama)

Flow karyotype analysis is suitable for distinguishing between isolated mammalian chromosomes. To assign structures and functions of human gene, we tried to sort chromosomes isolated from human lymphocytes by using Cell Sorter (Coulter Epics V).

We carried out the sort of chromosomes according to the method of Young *et al.*[1] Lymphocytes prepared from human blood by Ficoll-Conray method were added and grown in RPMI 1640 medium with 10% FCS and 0.005 μl/ml PHA-P for 48 hr. The metaphase were arrested with 0.32 μg/ml colcemid and the cultures were incubated for additional 14–16 hr. The fraction of mitotic cells obtained from this procedure was usually about 15%. Approximately 10^7 cells were swollen in 0.075 M KCl for 30 min at 0°C and they were treated by polyamine-containing buffer and then resuspended in 1–2 ml of polyamine buffer containing 0.1% digitonin. The chromosomes were released from the cells by passing the suspension through a 26-gage needle 5 or 6 times. After isolation, the chromosomes were stained at a final concentration of 25 μg/ml ethidium bromide as the DNA specific dye. The chromosomes were analysed by using a Cell Sorter with laser power of 1.2 W at 488 nm. The resulting chromosomal fluorescence was projected onto a photomultiplier attached to the Cell Sorter. The human chromosomes were resolved into about 14–15 peaks. This pattern was almost similar to that of Young.[1] Due to the polymorphism of the human chromosomes, flow karyotype patterns from different persons were not always same. It will be possible to construct the human chromosomal DNA libraries by sorting specific chromosome such as X, 22-chromosome. Such libraries will be available to study genetic diseases and to make artificial chromosomes in future.

1) Sillar, R. and Young, B.D. 1981. *J. Histochem. Cytochem.* **29**: 74–78

A34. 組み換え DNA 技法によるヒト染色体の研究・予報：中堀 豊・中込弥男（遺伝研・人類遺伝）. **Study of Human Chromosomes Using the Technique of Recombinant DNA:** Yutaka NAKAHORI andYasuo NAKAGOME (Natl. Inst. Genet., Mishima)

ゲノム DNA 中の繰り返し塩基配列は，制限酵素切断後の電気泳動によりバンドとして観察される．制限酵素 HaeIII による切断にて生ずるバンドのうち，3.4 kb と 2.1 kb の DNA 断片は男性に特異的に観察され女性には見られないので，Y 染色体上にある繰り返し配列だとされている．この 3.4 kb の Y 染色体特異 DNA を BamHI linker を用いて大腸菌のプラスミド pBR322 に組み込み，クローニングを試みた．構造異常による DNA 量の変化に基づいて特定の染色体またはその一部を分画分取（sorting）し，ラムダファージに組み込むことにより単一染色体由来の遺伝子ライブラリーを樹立する試みにつき，研究の計画と現在の状況を示した．

A35. Two Glucose 6-Phosphate Dehydrogenase Variants Found by the Screening Test for the Inhabitants in Ibaraki, Japan: Takeshi KAGEOKA (Inst. Clin. Med., Univ. Tsukuba, Ibaraki)

The screening test for glucose 6-phosphate dehydrogenase (G6PD) deficiency was performed using Beutler's spot test and six hundred fifty male blood samples submitted to the division of the blood transfusion in Tsukuba university hospital. The blood samples were centrifuged at 3,000 rpm for 10 min, then they were adjusted to 30% of hematocrit with addition of saline to make the hemoglobin concentration uniform on the spot. Two cases with reduced enzyme activity were found. Characterization of the two G6PD variants was carried out using partially purified G6PD according to the methods recommended by WHO Scientific Group. The inhibition constant (K_i) for the NADPH was measured by the method of Yoshida. All the substrate, substrate analogues and nucleotides were purchased from Boehringer-Mannheim, except for 2 deoxy-G6P and deamino-NADP, which were Sigma. The characterization was measured in a Beckman DU-8 recording spectrophotometer. The incidence of G6PD deficiency in the Ibaraki inhabitants was 0.3% and low as in Yamaguchi and Hiroshima.

Case 1; (M.H.) 23 years old man. 17% of normal enzyme activity; normal K_m G6P (45 μM): high K_m NADP (23 μM); normal K_i NADPH (23 μM); normal 2 deoxy-G6P utility (3.7% of G6P); slightly decreased deamino-NADP utility (38.5% of NADP); normal heat stability; sharp peak at pH 8.0 for pH optimum; slow electrophoretic mobility (on phosphate buffer pH 7.0, 80% of normal; on TEB buffer pH 8.6, 85% of normal). Case 2 (G.H.) 31 years old man. 38% of normal enzyme activity; slightly decreased K_m G6P (24 μM); normal values of K_m NADP (5.1 μM), K_i NADPH (27 μM), 2 deoxy-G6P utility (4% of G6P), and deamino-NADP utility (45% of NADP); normal heat stability; normal pH optimum; fast electrophoretic mobility (on phosphate buffer pH 7.0, 111% of normal; on

TEB buffer pH 8.6, 108% of normal). Family studies for case 1 and 2 revealed that both mother and sister were heterozygote. The both cases had no hemolytic episode as history. The case 1 was evaluated to be a unique variant.

A36. Hemoglobin G Waimanalo, an α-Chain Variant Found in Japanse Family: Jun SUGIHARA, Eisuke YOKOTA, Masaaki KAGIMOTO, Yoko OKAZAKI, Yasushi NAITO, Toshikazu MATSUO, Takashi IMAMURA and Toshiyuki YANASE (1st Dept. Med., Kyushu Univ., Fukuoka)

The propositus was a 40-year-old Japanese man with normal hematological parameters. The isopropanol test was negative for unstable hemoglobins. The thin-layer starch gel electrophoresis at pH 8.6 detected a hemoglobin variant that migrated slower than Hb A. The relative mobility was −4.88 when calculated on a cellulose acetate sheet. The hemoglobin variant, Hb A_2 and Hb F comprised 24.1%, 2.1% and 1.1% of the total hemoglobins, respectively. Abnomal component was isolated by DE52 chromatography. Oxygen binding properties of the variant hemoglobin was identical with those of Hb A. It was apparent that the normal αT9 peptide was absent in the map of the variant, while a new peptide was present. The amino acid composition of the altered αT9 separated by high performance liquid chromatography was identical with that of normal αT9. The substitution of an asparagine for an aspartic acid residue was determined by the use of Partridge and Davis' preferential cleavage of aspartic acid with 0.25 M acetic acid. While there are aspartic acid residue at positions 64, 74, 75 and 85 in Hb A, there was no cleavage at residue 63 which in Hb A precedes 64 aspartic acid. The peptides from aminoethylated β-chains were identical with those of the normal when examined by high performance liquid chromatography and by peptide mapping on paper support media. As there was no cleavage between residues 63 and 64, we conclude that the substitution must be an aspartic acid residue at the 64th position of the α-chains. This variant is, therefore, identical with Hb G Waimanalo.

A37. Abnormal Hemoglobin in Takamatsu District: Kazuo HIDAKA, Iwao IUCHI, Shunichi SHIMASAKI (Dept. Biochem., Kawasaki Med. Sch., Kurashiki) **and Minoru KUWASHIMA** (Kagawa Pref. Cent. Hosp., Takamatsu)

A hemoglobinopathic survey in Takamatsu area was continued for the past one year in the individuals totaling 7,500 since our first summarized report. Six fast-moving abnormal hemoglobins were detected; a half of them was referred to α chain anomaly and the other β chain anomaly. Two variants of the α chain anomaly were revealed to be Hb Ube-2 (α68 Asn→Asp) by the analyses of HPLC of the tryptic digest of α chain and sequential

degradation of aberrant peptide. Therefore, these two variants were the 2nd and 3rd instances of Hb Ube-2 in this area. No consanguineous relationship among these families was clarified. Two carriers of the β chain anomaly were detected at separate date. However, the family study revealed a kinship between the two carriers. The results of structural analysis of the variants showed a replacement of β120 Lys→Gln. Therefore, they were the 12th and 13th instances of Hb Takamatsu in eleven families. Both of the carriers of Hb Ube-2 and Hb Takamatsu demonstrated neither clinical symptoms nor hematological abnormalities and indicated normal oxygen affinity and stability as seen in previous instances. It is noteworthy that six carriers of Hb Ube-2 have already been discovered sporadically all over Japan. On the other hand, the distribution of Hb Takamatsu is localized in a rather small restricted area as an endemic Hb variant. The incidence of Hb variant in this area was 0.08% This figure is higher than that (0.03%) of any other parts of Japan.

A38. 異発生性男性半陰陽の1例：康　明照・本田正之（宮崎県立延岡病院・産婦人），**広田昭三**（同・外科），**康　維邦**（高雄市立民生病院・産婦人）．**A Case of Dysgenetic Male Hermaphroditism:** Akiteru YASUDA, Masayuki HONDA (Dept. Obst. Gynec., Miyazaki Provincial Nobeoka Hosp., Nobeoka), Shozo HIRODA (Dept. Surg. Miyazaki Provincial Nobeoka Hosp., Nobeoka) and Korekuni YASUDA (Dept. Obst. Gynec., Kaohsiung City Min-Sei Hosp., Kaohsiung)

今回われわれは，嵌頓ヘルニアにて緊急還納手術中に異発生性男性半陰陽と診断された貴重な症例を経験したので報告す．症例：52歳，同胞る6人の第4子，男として認知された．同胞いずれも健常．血族結婚なし．小学校2年ころからときどき右ソケイ部ヘルニアが出現し，いつも還納できたが，S5, 2, 21 にヘルニアが出現し，今度は内容がいつもと違って硬く，還納できず，S57, 2, 23 に緊急入院となった．体格は男性型である．男子の二次性徴が認められ，性欲も存在する．性生活ほとんど正常と変わりがない．外陰部は完全に男性型，陰茎は正常，陰嚢発育は正常で左陰嚢には睾丸欠如，右陰嚢には睾丸と思われる腫瘤を触知，さらにその上方（右ソケイ部）に手拳大の腫瘤を触知した．右ソケイ部にて切開し，hernial sac を出したところ，その内容は子宮および左性腺であった．いわゆる hernia uteri inguinalis と考えられる．右性腺は虫垂手術のため腹壁に癒着．子宮につづいて膣があり，盲端になって終わっている．病理組織的に両側性腺ともに未分化の睾丸と思われる．卵巣は検索した限り，どこにも存在しなかった．染色体は 46,XY，性染色質は 0%，内分泌学的には高ゴナドトロピン状態を示し，血中テストステロン値は正常よりやや低い．性腺原発性障害が示唆される．尿中 17KS, 170HCS, 17KGS 値は正常男性闘にあった．本症の成因は，müller 管抑制ホルモンの欠如また不足によると思われる．

A39. 常染色体優性遺伝を示す Ruvalcaba 症候群家系：杉尾嘉嗣・松尾清巧・梶井　正（山口大・小児）. **A Family of Ruvalcaba Syndrome with Autosomal Dominant Inheritance:** Yoshitsugu SUGIO, Kiyosato MATSUO and Tadashi KAJII (Dept. Pediatr. Yamaguchi Univ. Ube)

Ruvalcaba 症候群は低身長，精神薄弱，細い曲がった鼻，太く短い指，その他の奇形を伴う多発性奇形症候群である．3 家系の報告があるが，その遺伝形式は決定していない．Ruvalcaba ら (1971) の報告した家系は，兄弟 2 人が罹患し，その母方の従姉妹 2 人が部分症状を呈したので X 連鎖性だとされた．Georgmaneanu ら (1978) の報告例は 7 歳男子で，その父が短指症を呈し，父子ともに t(13q14q) 型転座を有していた．Hunter ら (1977) の報告した家系は，3 世代，6 人が罹患し，常染色体性優性と考えられる．この家系では obligate carrier 4 人が罹患しておらず，したがって浸透率は低い．この家系の患者は craniosynostosis を呈し，Ruvalcaba らの記載した症候群とは異なる新しい症候群だと Hunter らは考えている．筆者らは 4 世代，9 人に本症（またはその部分症状）を認めた家系を経験した．発端者は 3 歳男子で，その母，妹（8 か月）の 3 人はほぼ本症の症状を満たす．そのほかに母方の祖父，その同胞（男 1 人，女 2 人），母方の曽祖父，その姉の計 6 人が本症の部分症状を呈した．したがって，さまざまな表現度を呈する常染色体優性遺伝形式をとる疾患だと考える．文献に報告されている 3 家系は，浸透率および表現度が低い常染色体優性遺伝に矛盾しない．したがって，本症は常染色体優性遺伝と考える．

A40. 乳児期に発症した毛細血管拡張性失調症：塚原正人・小林邦彦・梶井　正（山口大・小児），**益田道義**（山口大・2 病理）. **An Atypical Form of Ataxia Telangiectasia. Report of an Infant:** Masato TSUKAHARA, Kunihiko KOBAYASHI, Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med.) and Michiyoshi MASUDA (2nd Dept. Pathol., Yamaguchi Univ. Sch. Med., Ube)

経過：女児．新生児期から哺乳力・体重増加不良．2 か月から全身の褐色色素沈着，3 か月から発熱を伴う咳嗽発作を認め急性咽頭炎に罹患しやすかった．経過観察中 1 歳 1 か月時，発熱・下痢・無顆粒球症を認め 1 歳 2 か月時 pneumocystis carinii 肺炎で死亡．検査所見：末梢血リンパ球減少，IgA 低値，ヘモグロビン F 高値，PHA 刺激に対する末梢血リンパ球の無反応．骨髄染色体分析で染色分体型ギャップ・切断 0.06/細胞，皮膚培養線維芽細胞では染色分体型ギャップ・切断 0.33/細胞，X 線感受性を認めた．MMC 感受性は認めなかった．骨髄・線維芽細胞ともに染色体異常クローンは認めなかった．両親の末梢血リンパ球分析ではギャップ・切断の増加は認めなかった．剖検：小脳 purkinje 細胞の減少，胸腺低形成，Hassal 小体欠如，全身リンパ腺低形成，卵巣低形成，脳小血管の拡張，胸腺・肝・リンパ腺・心筋の毛細血管拡張を認めた．結論：毛細血管拡張性失調症は乳児期に発症することは珍しい．本例は毛細血管拡張性失調症の臨床所見（易感染性，IgA 低値，PHA に対するリンパ球反応低下，諸臓器の毛細血管拡張，胸腺低形成，小脳変性，X 線感受性）と Fanconi 貧血の臨床所見 (cáfe au lait spots を伴う全身の褐色色素沈着，汎血球減少，胎児性ヘモグロビン高値）を示した．他方，染色体の反応（X 線感受性の亢進，MMC 感受性正常）は毛細血管拡張性失調症に一致し，その非定型例だと結論した．

A41. A Case Report of Potter's Type 1 Cystic Kidney: Akira MATSUI, Norio AMADA, Tetsuya HOSHINO, Kikuo SUZUKI (Dept. Pediatr., Isesaki City Hosp., Isesaki), **Yutaka SUZUKI** (Dept. Pathol., Isesaki City Hosp., Isesaki), **Takeshi MATSUDA and Toshio NAKATANI** (Dept. Anat., Toyama Med. Pharm. Univ., Toyama)

The baby (I.H) was born on Sept. 10, 1981 at 36 weeks' gestation and weighed 2,650 g at birth. He was noted to have severe cyanosis over the entire body and apnea with bradycardia (55 beats/min). Apgar score was 3 both at one and five minutes after birth. He was admitted to the neonatal unit and was treated with IPPB through endotracheal incubation. His abdomen was markedly distended. Fist-sized tumors were palpated over the bilateral flank. The chest roentgenogram demonstrated atelectasis of both sides. He died at 2 hr after birth. An autopsy findings revealed bilateral enlarged sponge kidney (rt 150 g, lt 130 g) and incompletely dilated lungs of both sides. Histopathologically was observed polycystic kidney with minute cysts, elongated in the cortex and round or oval in the medulla, associated with mild increase of bile ducts in the liver. From these findings Potter's type 1 cystic kidney was confirmed.

A42. Prothrombin Tokushima: A Family Including a Double Heterozygote with Dysprothrombinemia and Hypoprothrombinemia: Akira SHIRAKAMI, Shigenori KAWAUCHI, Toshio SHIGEKIYO, Yujiro HIRAI, Shiro SAITO (1st Dept. Int. Med., Univ. Tokushima, Tokushima) **and Kazuo MIYOSHI** (Okinaka Mem. Inst. Med. Res., Tokyo)

In a family of Tokushima district, 3 individuals with congenital dysprothrombinemia (maternal side), 5 with congenital hypoprothrombinemia (paternal side) and one double heterozygote for both were found, each of which has never been reported in Japan. The proband, 10-year-old girl, was the double heterozygote, who was referred to us because of prolonged bleeding after extraction of teeth. In this patient, FII (prothrombin) activity was markedly decreased to 12%, but FII antigen was 42%, about half the value of normal individuals. In studies on family members, the mother of proband, her mother and her younger brother were found to have on average 60% FII activity and 90% antigen, showing the decrease in activity alone. These three individuals appeared to be heterozygous for congenital dysprothrombinemia. FII activity and antigen per single gene, and activity/antigen ratio were calculated to be 10%, 40% and 0.25, respectively, in this dysprothrombinemia, as compared to 50%, 50% and 1.0 in normal. The father of the proband, his elder sister, his younger brother and his niece had FII activity and antigen of about half the value of normal individuals, 58% and 48% on average, respectively. Each of the five appeared to be heterozygous for congenital hypoprothrombinemia with 0% activity and 0% antigen. The

proband, a double heterozygote, showed FII activity and antigen pattern expressed by single gene of dysprothrombinemia. The activity in the proband was 12% by one-stage method but 22% by two-stage method, showing some delay in thrombin formation. However, calcium-binding site appeared normal, since the adsorption by $BaSO_4$ was normal. The prothrombin activity of the proband was 48% by Echis venom, a value corresponding to that of the antigen, and 14% by Taipan venom, a value corresponing to that obtained by the one-stage method, each of which suggested the abnormality of the cleavage site by FX_a. In the literature, 10 families of simple congenital dysprothrombinemia, 17 families of simple congenital hypoprothrombinemia and 2 families, including double heterozygotes for both, have been reported. However, details of similarities and differences in characteristics of these dysprothrombins have not been elucidated. Prothrombin Tokushima is proposed for this dysprothrombin.

A43. A Case of Lipoid Adrenal Hyperplasia (Prader Syndrome) with i(Xq) Type Turner Syndrome: Akira HATA, Yozo ICHIBA (Dept. Pediatr., Natl. Okayama Hosp., Okayama), **Michiko NAKASHIMA and Shinichiro UEHARA** (Dept. Pediatr., Himeji St. Maria Hosp., Himeji)

The patient was born at 42 weeks of gestation as the first child of healthy and unrelated parents after an uneventful course of pregnancy and delivery. Her birth weight was 2,560 g. She was referred to NICU because of generalized pigmentation, but had no other physical findings. At about 10 hr after birth, we started intravenous fluid therapy and phototherapy for hypoglycemia (10 mg/dl>) and hyperbilirubinemia (10.2 mg/dl). At 7th day hyponatremia (120 mEq/liter) and hyperkalemia (8.1 mEq/liter) were developed and we initiated to give hydrocortisone, Florinef and NaCl suspecting the diagnosis of Prader syndrome. After treatment was begun, she made a quick recovery and showed good weight gain. Before treatment serum ACTH level was over 800 pg/ml and serum cortisol level was 2.4 μg/dl. LH-RH test for ruling out adrenal hypoplasia showed overreaction of LH and FSH, indicating she suffered from Prader syndrome. Cytogenetic study was carried out since patients of Prader syndrome show phenotypically female regardless of genetic sex. The results of that was 46,X,i(Xq). Combination of Prader syndrome and i(Xq) type Turner syndrome may be only fortuitous but we think this association is too rare to draw such a conclusion.

A44. Unidentified Type of Achondrogenesis in Two Siblings: Toyoshi TSURUTA, Naohiko TAKI, Ikuo TSUNODA, Hirokuni OZAWA and Tomoko HASEGAWA (Dept. Orthop., Univ. Mie, Mie, and Natl. Tachikawa Hosp., Tokyo)

Two siblings with "achondrogenesis" are reported. The patients were children of healthy, unrelated parents. Macroscopic appearance of the still-borne babies resembled those of classical achondrogenesis. However, roentgenograms revealed well-ossified calvaria and vertebral bodies; pelvic bones were hypoplastic, but well-ossified and the greater sciatic notch was not narrowed as in case of thanatophoric dysplasia; long bones of the extremities were extremely short and broad, and spurs were observed on their metaphyses. As these X-ray findings are clearly different from those of achondrogenesis Type I or II, these cases seem to represent a "new" clinical entity.

A45. Decreased Colony-Forming Ability after UV-Irradiation in Four Patients from Three Families with Basal Cell Nevus Syndrome: Tomoko HASHIMOTO, Hiromi SAKAMOTO, Hide-aki CHIYO, Chiharu TAKADA, Patricia LOPETEGUI, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med.), **Takahiko SUKENAGA** (Dept. Radiol., Hyogo Coll. Med., Nishinomiya), **Shigekazu SHIMAOKA, Chuzo MORIMOTO and Rikiya SHIRASU** (Dept. Oral Surg., Osaka Cent. Univ., Osaka)

The basal cell nevus syndrome (BCNS) is an autosomal dominant genetic disorder with multiple basal cell carcinomas, multiple odontogenic cysts, skeletal anomalies, pits of palms and soles, and laminar calcification of the falx cerebri. Seven patients with BCNS were found in three families. None of them had skin cancers, but one of them had died of medulloblastoma in his infancy. The skin fibroblasts derived from the four patients (BCNS-1NI, 2NI, 3NI and 4NI) were studied. These fibrobalsts exhibited normal sensitivity to γ-irradiation and to bifunctional mitomycin C, but hypersensitivity to ultraviolet (UV) light, as measured by colony-forming ability. D_0 value of the fibroblasts after UV-irradiation was 2–3 J/m^2 in BCNS cells, but 5–6 J/m^2 in control cells and 0.5 J/m^2 in xeroderma pigmentosum (complementation group A) cells. Unscheduled DNA synthesis after UV-irradiation was normal. Thus, BCNS appeares to be the only autosomal dominant disease that presents hypersensitivity to UV-irradiation.

A46. リンパ芽球の細胞融合によるメープルシロップ尿症の遺伝的異質性の検討：陣野吉広・赤星　泉・松田一郎・甲木孝人* (熊本大・医・小児, *微生物). **Analysis of Genetic Heterogeneity of MSUD by Fusion of Lymphoblastoid Cells:** Ichiro MATSUDA *et al.* (Dept. Pediatr., Kumamoto Univ., Kumamoto)

MSUD 患者由来のリンパ球を EB ウイルスによって樹立した培養リンパ芽球を用いて，ポリエチレングリコールによる細胞融合を行い，MSUD の遺伝的異質性を検討した．5 cell lines の可能な組み合わせ 10 通りについて検索した結果，heterokaryons を含む細胞集団の酵素活性の，parent cell の homokaryons を含む二つの細胞集団の活性の和の 1/2 に対する比は 0.7～2.6 にわたった．この結果から，MSUD は少なくとも二つ以上に分類される遺伝的異質性を示した．また計算によって heterokaryon のみの酵素活性を求めたとき，きわめて興味ある特徴が認められ，相補のメカニズムが異なる可能性が示唆された．cloning で hybrid clone を検索することによって，相補のメカニズムが明らかにされるかもしれない．

A47. Carrier Detection by Protein Loading in Ornithine Transcarbamylase Deficiency: H. YAMAMOTO, Y. FUKUDA, Y. SAWADA, Y. HASE, T. TSURUHARA, T. OURA (1st Div. Pediatr., Child. Med. Cent. Osaka City, Osaka), **A. MATSUSHIMA and T. ORII** (Dept. Pediatr., Gifu Univ., Gifu)

Ornithine transcarbamylase deficiency is an X-linked recessive disorder which usually causes lethal hyperammonemia in affected male infants. Carrier females have varying amounts of residual enzyme activity and show variable clinical signs depending upon the proportion of functionally active X chromosomes which carry the mutant gene. We examined urinary orotic acid excretion and plasma ammonium concentration after protein loading in the parents, two sisters, maternal grandmother and maternal aunts of an OTC deficient girl in order to detect asymptomatic heterozygotes.

They ingested a breakfast providing natural protein of 1 g/kg body weight. Orotic acid was measured in the urine before, and in 4- or 6-hr collection after the protein load using the modification of the method of Adachi *et al.* Plasma ammonium concentrations were determined at one-hour interval after protein load for 4 hr in some of them. The mother and one younger sister developed hyperammonemia up to 95 μg/dl 2 or 3 hr after load. In the father and another younger sister, plasma ammonium concentrations remained within normal range during 4 hr. Prominent orotic aciduria after load was noted in the mother and in one sister when she was tested at 18 month old. It was noteworthy that there was no rise in orotic acid excretion when the same test was carried out at her age of one month. No significant increase in urinary orotic acid was noted in the father, another sister and the maternal aunts. The maternal grandmother, though being an obligate carrier, also showed no elevation of orotic acid excretion. Orotic acid determination after protein load was thought to be a useful but not yet a complete method for the detection of asymptomatic carrier of OTC deficiency.

A48. A Family with Silent Type of Serum Cholinesterase Variant and Hereditary Cerebellar Ataxia: **Hisaomi KAWAI, Hiroshi NISHINO, Shinji MIURA, Hiroshi FUJIMOTO, Kenjiro MASUDA, Shiro SAITO** (1st Dept. Int. Med., Tokushima Univ., Tokushima), **Nozomu OGASAWARA** (Takamatsu Red Cross Hosp., Takamatsu) **and Kazuo MIYOSHI** (Okinaka Mem. Inst. Med. Res., Tokyo)

Of five children (2 males, 3 females) born of apparently healthy parents of first cousins, the third daughter aged 24 (the proband) had serum cholinesterase (ChE) deficiency and cerebellar ataxia, and the first son aged 30 was heterozygous for ChE deficiency, while the second son aged 28 had cerebellar ataxia and selective IgA deficiency. The activity of serum ChE was markedly decreased to 0.02 ΔpH in the proband, a homozygote. The activity was 0.55, 0.77 and 0.65 ΔpH in the father, mother and the first son, respectively, which were suggestive of heterozygotes. However, it was normal, 1.19 ΔpH, in the second son with cerebellar ataxia. Serum ChE isozyme pattern of the proband showed very weak staining of five bands, C_1–C_5. Staining of the bands in the father, mother and the second son was decreased. Susceptibilities to inhibition by dibucaine and fluoride were not detectable in the proband, but were normal in the father and the first son. Both the proband and the second son developed gait distrubance around 20 years of age, suggestive of hereditary cerebellar ataxia. Brain CT revealed cerebellar atrophy. The second son was associated with selective IgA deficiency (IgA was 8 mg/dl). This is a family of silent type of serum ChE variant with one homozygote and three heterozygotes. Reports of silent type of serum ChE variant are still rare (only 9 families) in Japan. The coexistence of ChE deficiency and cerebellar ataxia in the proband and of IgA deficiency and cerebellar ataxia in the second son can be explained by the association of chromosomes with variant genes for each disorder. However, the presence of three types of rare abnormalities among siblings born of a consanguineous marriage suggests the existence of some reason in the dissociation and association of genes and the mechanism of their expression.

A49. Adenosine deaminase (ADA) 欠損症と ADA 活性について：三山隆司・角谷憲史・崎山幸雄・荒島真一郎・松本脩三（北大・医・小児），**永井文作**（帯広厚生病院・小児）．**ADA Activity in ADA Deficiency:** S. MATSUMOTO *et al.* (Dept. Pediatr., Hokkaido Univ., Sapporo)

adenosine deaminase (ADA) 欠損による重症複合免疫不全症は，本邦において非常に稀な疾患である．われわれが新たに経験した本症（6 か月女児）について，患児および家系内での ADA 活性を測定したところ若干の知見を得た．

患児赤血球の ADA 活性は，正常対照が 823.1～984.7 mU/g Hb であるのに対し，53.7 mU/g Hb と低下していたが約 6% の残存活性が認められた．残存 ADA についてアデノシンに対する K_m，至適 pH，熱安定性を検討したところ，正常 ADA に比べ熱安定性のみが異なり，患児 ADA は熱に対して安定であった．培養皮膚線維芽細胞でも ADA 活性の低下が認められたが，やはり約 6%

の残存活性があった．治療の目的で ^{60}Co 照射した凍結赤血球 90 ml を輸注した．輸注後の赤血球中の ADA 活性の変化をみると，輸注 24 時間後より約 300 mU/g Hb までの活性上昇が認められたが，この値は期待活性上昇値より約 30% 増で，内因性 ADA 活性物質の存在が示唆された．これらのことから，本例での ADA の異常は触媒作用を有する catalytic unit の mutation によるものであることが示唆された．家系内検索では両親とも赤血球 ADA 活性の低下を認め heterozygote であり，遺伝形式としては常染色体劣性遺伝が示唆された．また本症の出生前診断を前提に，正常羊水での ADA 活性を検討した．培養羊水細胞では ADA 活性は認められ出生前診断に役立つが，羊水中でも弱いながら ADA 活性が認められ，出生前診断の一助となると考えられた．

A50. Characterization of a Low Molecular Weight Immunoglobulin G Appearing in a Case of Multiple Myeloma: Akihiko HOSHI (SRL, Tokyo), **Tomotaka SHINODA** (Dept. Chem., Tokyo Metropol. Univ., Tokyo), **Ikunosuke SAKURABAYASHI, Tadashi KAWAI** (Jichi Med. Coll., Tochigi), **Kunito IWABUCHI** (Seiwa Hosp., Takiwagi) **and Chuichi ITO** (Iwate Med. Coll., Iwate)

A low molecular weight IgG has been isolated from the urine of a patient with multiple myeloma by combination of ammonium sulfate fractionation, DEAE-cellulose column chromatography, and gel filtration with Bio-gel A-5 m or with Sephadex G-200. The specimen (NIG-91) existed in a uniform molecular form with a molecular weight of 30,000 daltons when examined in SDS-disc electrophoresis, suggesting that it was a monomer. It reacted only with an antiserum specific for IgG, but did not give any reaction against other antisera specific for normal human serum proteins. The NH_2-terminal sequence was found to be Thr-Pro-Glu-, suggesting that it initiated from the residue 256 of the γ 1 heavy chain. The C-terminal amino acid was identified as Gly which was identical with that of the C-terminal of normal γ heavy chain. Although the sequence analysis is rather limited at present, the data strongly suggest that the specimen is a fragment corresponding to the region initiated from the residue 256-Thr and ended with Gly, the C-terminal residue of the normal γ chain. The amino acid composition also supports the above conclusion.

A51. Two Siblings with β-Galactosidase-Neuraminidase Deficiency (Galactosialidosis) Who Have Unusual Clinical Courses: Eiji TAKEDA, Yasuhiro KURODA, Tomoko TOMITA, Hideaki KOBASHI, Michinori ITO, Toshiyuki WATANABE, Kenji TOSHIMA, Toshiaki HASHIMOTO and Masuhide MIYAO (Dept. Pediatr., Tokushima Univ., Tokushima)

Unusual cases with β-galactosidase-neuraminidase deficiency (galactosialidosis) were reported here. The patients were 5 and 8 years old female siblings. Their parents were not consanguineous. Blue macular skin lesions appeared on whole bodies at 2 to 3 months

after birth in these patients. At the age of 3 and 4 years, they complained visual disturbance and cherry-red spots were observed. Younger sibling became aware of hearing loss at 4 years of age. The siblings were admitted to our hospital for a precise examination at the age of 5 and 8 years. The major clinical findings of these patients included blue macular skin lesions, coarse facial features, dysostosis multiplex, hearing deficit, hyperactivity of deep tendon reflex, joint contracture and atrophy of hand muscle. However, myoclonus, cerebellar sighs and seizure attack were not observed. Psychomotor development was normal. Both activities of β-galactosidase and neuraminidase in white blood cells and cultured skin fibroblasts markedly decreased and urinary excretion of sialic acid-rich oligosaccharides increased in these patients. Galactosialidosis is still a equivocal disease entity because of marked variations of individual symptoms. In previously reported cases, visual loss, cherry-red spots, myoclonus and cerebellar sighs were frequently observed. The visual loss as a initial symptom in most cases was found after 7 years and other symptoms were later than 10 years of age in this disease. In contrast, myoclonus and cerebellar sighs were not observed and visual disturbance and cherry-red spots were found at the age of 3 and 4 years in our patients. Thus, our cases are considerably different from the patients reported previously. Therefore, it is necessary to follow up their clinical course, because it may be thought that our patients are a variant form or early diagnosed cases of galactosialidosis.

A52. Suppression of Sister Chromatid Exchange in Bloom's Syndrome Fibroblasts upon Fusion to Enucleate Cytoplasts from Chinese Hamster Cells: Michihiro C. YOSHIDA (Chromosome Res. Unit., Fac. Sci., Hokkaido Univ., Sapporo) **and Toyozo SEKIGUCHI** (Natl. Cancer Cent., Tokyo)

The effect on the rate of sister chromatid exchanges (SCEs) in cultured fibroblasts from a patient with Bloom's syndrome (BS) by fusion with enucleate cytoplasts from Chinese hamster (wg3h) cells was studied. The high rate of SCE was found to be fully corrected in BS chromosomes in fusion products (cybrids) between hamster cytoplasts and BS fibroblasts. The rate of SCE in the cybrids was nearly identical with that of SCE found in normal human diploid fibroblasts or Chinese hamster wg3h cells. This suppression effect was maintained for 10 days after fusion. No suppression of the frequency of SCE was observed in the BS cells when these were co-cultivated with Chinese hamster cells. These results indicate that cytoplasm from Chinese hamster has the ability to rescue the Bloom-specific increase in SCE and may contain corrective factor(s) for SCE formation in BS cells. However, cybrid cells following further subcultivation and cybrid clones isolated in the HAT+chloramphenicol selective medium showed the high rate of SCE similar to that of parental BS fibroblasts, indicating that corrective factor(s) in cybrids might be lost

or diluted out through the cell division. Although the corrective factor(s) was unstable in cybrid, the origin and nature of cytoplasmically derived corrective factore(s) remain to be determined. Such studies are now in progress.

A53. Analysis of Bloom Syndrome Sister Chromatid Exchanges by Three-Way Differentiation in Bromodeoxyuridine-Substituted Chromosomes: Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Kochi)

Sister chromatid exchanges (SCEs) during first (SCE_1), second (SCE_2) and third (SCE_3) cell cycles were analysed with three-way differentiation technique of sister chromatids in normal and Bloom syndrome (BS) cells. In normal cells, the mean frequencies of SCE_1, SCE_2 and SCE_3 were 2.44 ± 0.11, 2.88 ± 0.15 and 5.08 ± 0.14, respectively. The ratio of SCE_1 to SCE_2 was approximately 1 : 1, while the value of SCE_3 was significantly higher than that of SCE_1 and SCE_2. In contrast, though BS SCE_1 (3.28 ± 0.13) was only slightly higher than normal (2.44 ± 0.11), BS SCE_2 and SCE_3 were significantly higher than SCE_1 and were 63.32 ± 2.13 and 73.08 ± 2.15, respectively. The combined frequencies of SCE_1 and SCE_2 correspond almost exactly with those obtained at second mitoses labeled with BrdU for two cell cycles in both normal and BS cells. This finding agrees with that observed in endomitotic analysis of single and twin SCE. The present finding strongly supports our previous notion that spontaneous SCE levels in BS cells were normal range and most of BS SCEs occur when BrdU-containing DNA is used as the template for replication.

A54. Induction of Sister Chromatid Exchanges in Human Lymphocytes. (1) Cell Stage Dependency and Effect of Blood Donor's Passive Smoking: Kanehisa MORIMOTO, Kunihiko MIURA, Mayumi SATO, Harumi KIKUCHI and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

To see the cell stage dependency of chemical induction of sister chromatid exchanges (SCEs), lymphocytes were treated with increasing concentrations of mitomycin C (MMC), or 4-nitroquinoline-1-oxide (4NQO) at the 0th, 24th, or 48th hr after stimulation of cultures. The results show that treatment at the 24th hr is the most effective in inducing SCEs. The induction of SCEs was apparently smaller when cells were treated with very high concentrations of the chemicals, because of differential sampling of less severely damaged cells. A longer than 72 hr incubation of cells, thus, resulted in a larger induction of SCEs.

Many investigators have described strong correlations between the frequency of sister chromatid exchanges (SCEs) in lymphocytes and health situations and/or living habits. Public health attention has recently been paid to the ill health effect of passive smoking. We showed before that the SCE frequency in lymphocytes from smokers increases accord-

ing to the equation: $y=6.4+0.51x$; y, SCEs per cell; x, number of cigarettes smoked per day. We have now investigated the effect of passive smoking on the SCE frequency in lymphocytes from persons that have been working in the environment where many people smoke usually, *i.e.*, *cafe*. The spontaneous SCE frequencies in passive smokers were about the same as those in cells from non-smokers. However, lymphocytes from both passive and active smokers had an increased frequency of SCEs compared to those from non-smokers when exposed to mitomycin-C (MMC) during incubation.

A55. Induction of Sister Chromatid Exchanges in Human Lymphocytes. (II) Induction of Resistance to Alkylation Damage: Mayumi SATO, Kanehisa MORIMOTO and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

When the bacteria and mammalian cell lines are exposed to very low nontoxic doses of an alkylating agent, the adaptive DNA pathway which enables the bacteria and mammalian cells to resist the lethal or mutagenic effects of further alkylation damage is induced. We have performed experiments to see whether repeated pretreatments of human lymphocytes can render the cells resistant to the induction of sister chromatid exchanges (SCEs) by alkylation damage. Human lymphocytes were pretreated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) at 0, 5, 10, 25, 50 ng/ml once every 6 hr for 72 hr starting from the 24th hr after PHA stimulation. After the last pretreatment, cells were challenged with 4 μg/ml MNNG and then treated with BrdUrd for two complete cell cycles. When 10 ng/ml MNNG pretreatment was employed, SCEs were induced by the challenge treatment. We performed further examinations to see if there was cross reaction between adapting with MNNG and challenging with other chemicals which would produce different DNA damage, such as ethyl-nitrosourea (ENU), mitomycin-C (MMC), or 4-nitroquinoline-1-oxide (4NQO). We found that lymphocytes pretreated with 10 ng/ml MNNG for 72 hr showed markedly reduced production of SCEs when challenged by ENU or 4NQO but did not do so when exposed to MMC. This data indicates that repeated pretreatment with MNNG can induce repair enzymes in cells (possibly methyl- or ethyltransferase) which remove the alkylation damage produced by MNNG, ENU, and 4NQO, as found in *E. coli*, and the mammalian and human cell lines.

A56. Induction of Sister Chromatid Exchanges in Human Lymphocytes. (III) Chemical Sensitivities in Cells from Familial Polyposis Coli: Kunihiko MIURA, Kanehisa MORIMOTO, and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

Peripheral lymphocytes from three patients with familial polyposis coli (FPC) including one patient with Gardner sign, and two healthy unrelated adults were studied for sister

chromatid exchanges (SCEs) in cultures treated with mitomycin C (MMC), 4-nitroquinoline-1-oxide (4NQO), and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Cells were treated for 1 hr before phytohemaggulutinin (PHA)-stimulation (pulse treatment) or for 72 hr during the whole culture (continuous treatment). In any culture, SCE induction ratios in FPC cells to normal ones were less than two and no significant difference in the SCE induction was observed between FPC and normal cells. Previous studies have reported FPC cells to be hypersensitive to MMC, 4NQO, or MNNG in colony formation, or in the induction of chromosomal aberrations. Three possibilities were discussed to explain the discrepancy in chemical sensitivities in FPC cells between previous reports and the present study: i) Heterogeneity in sensitivities or possible sub-groups in patients with this syndrome, ii) differences in sensitivities in stages in the natural history of this disease, and, iii) possible differences in the induction mechanisms between SCEs and chromosomal aberrations or cell death when treated with DNA-damaging chemicals.

A57. Sister Chromatid Exchanges, Chromosome Aberrations and Proliferative Kinetics in Tuberous Sclerosis Lymphocytes: Atsushi IESHIMA, Kousaku OHNO and Kenzo TAKESHITA (Div. Child Neurol., Tottori Univ., Yonago)

Skin fibroblasts from tuberous sclerosis (TS) have been reported to have hypersensitivity to γ-irradiation or γ-ray like chemicals; *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG). We investigated the frequencies of chromosome aberrations and sister chromatid exchanges (SCE), and cell cycle kinetics in MNNG-treated lymphocytes from 7 patients with TS and 7 controls. All patients were sporadic cases, 4 males and 3 females, aged from 6 years to 36 years of age. Controls were healthy subjects, 3 males and 4 females, age-matched with TS subjects. Lymphocytes were cultured for 72 hr in the medium containing 5 μg/ml BrdU, added MNNG (0 μg/ml, 1 μg/ml, 2 μg/ml, 3 μg/ml) for last 36 hr. Sister chromatids were differentially stained by FPG methods. Chromosome aberrations were examined in 50 cells of first division (M1). SCE frequencies were counted in 30 cells of second division (M2). One hundred metaphases were divided into first (M1), second (M2) and third or later division (M3). The frequencies of chromatid type aberrations (breaks and interchanges) and chromosome type aberrations (breaks, rings and fragments) were not different between TS and control group. The frequencies of SCE in TS were 5.9 ± 0.77 (untreated), 18.1 ± 8.01 (MNNG; 1 μg/ml), 31.4 ± 4.97 (MNNG; 2 μg/ml) and 40.6 ± 6.11 (MNNG; 3 μg/ml), whereas those of SCE in control group were 6.5 ± 0.92 (untreated), 16.5 ± 1.26 (MNNG; 1 μg/ml), 34.0 ± 7.69 (MNNG; 2 μg/ml) and 40.6 ± 6.11 (MNNG; 3 μg/ml), respectively. There were no significant differences between both groups. Cell cycle kinetics were not different between TS and control group in each MNNG concentration.

A58. Sister Chromatid Exchanges in Lymphocytes from Patients with Tuberous Sclerosis: Sumio IIJIMA, Makoto HIGURASHI, Tatsuya TAKESHITA (Dept. Health Sci., Med. Univ. Yamanashi, Yamanashi), **Masaya SEGAWA** (Segawa Neurol. Clin. Child.) **and Masuko FUNAHASHI** (Tama Habilitat. Clin. Child Neurol., Tokyo)

Lymphocytes from four normal controls and from four patients with tuberous sclerosis (TS) were studied for sister chromatid exchanges (SCEs) and cell cycle kinetics when they had undergone first, second, third or more divisions in mitomycin C (MMC; 0, 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7} M)-treated cultures (72 hr culture). Two hundred metaphase cells were scored for cell cycle kinetics, and 30 consecutive second-divison metaphases were scored for SCEs per point per person. Frequencies of spontaneous SCEs in controls and TS cells were 8.4 ± 0.8 and 7.5 ± 0.5, respectively. They were within the normal range. MMC treatments of TS and normal cells led to a clearly dose-related increase in frequencies of SCEs. However, there were no significant differences between frequencies of SCEs in TS and normal cells in all MMC concentrations examined.

Analysis of cell cycle kinetics by the sister chromatid differential staining method revealed that there were no remarkable differences between cell cycle kinetics of TS cells and those of normal cells. These data suggest that TS cells proliferate at almost the same rate as normal cells. In summary we have demonstrated that TS lymphocytes had almost the same proliferative kinetics and SCE frequencies as normal cells both in untreated cultures and when exposed to MMC.

(Supported by a grant from the Ministry of Health and Welfare of Japan.)

A59. H-Y Antigen in Patients with Abnormal Sexual Differentiation: Hiromi SAKAMOTO, Miyako YABUKI, Osamu MIKAMI, Hide-aki CHIYO, and Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med., Nishinomiya)

Detection of H-Y antigen in patients with abnormal sexual development is being carried out in our laboratory. Goldberg's method was used to detect H-Y antigen. However, this method is rather troublesome, so now the following method is being employed: H-Y antibody induced in mice is absorbed with the patient's WBC, then the cytotoxic ability of the supernatant is checked using Raji cells as target cells. The results we obtained with either method were the same. Up to now we have studied 16 patients in which the gonad histology is clear. Regardless of their sex chromosome constitution, there was strict correlation between expression of H-Y antigen and the type of gonad that developed in all cases but two. Of these, one was a Turner syndrome in which H-Y antigen was expressed but had a streak gonad. The other was a patient in which, although he has

testis, H-Y antigen is not expressed. Based on these results the possible mechanisms of expression of H-Y antigen are discussed.

A60. 遺伝性リソゾーム蓄積症の疾患別頻度に関する研究—出生前診断の疾患別頻度を中心にして—：大和田操，守屋百子，北川照男（日大・医・小児）．**A Study on Incidence of Lysosomal Storage Diseases—Data from Prenatal Diagnosis for Inborn Errors of Metabolism—:** M. OWADA, M. MORIYA and T. KITAGAWA (Dept. Pediatr., Nihon Univ. Sch. Med., Tokyo)

リソゾーム蓄積症の頻度には人種差が認められるが，わが国における各疾患の頻度については不明な点が多い．1982年に厚生省心身障害研究小児慢性疾患研究班が行った調査では，700例の代謝性蓄積症が報告され，そのうち400例がリソゾーム蓄積症であったという．また，同班が行った，先天性代謝異常症の出生前診断に関する調査では，127例の報告があり，そのうちリソゾーム蓄積症の出生前診断が108例で全体の85%を占めている．一方，われわれの教室では，1972年から1983年までに45例の先天性代謝異常症の出生前診断を行ったが，そのうちリソゾーム蓄積疾が43例で95%を占め，Gaucher病14例，Tay-Sachs病10例，I-cell病9例と，これら3疾患の出生前診断が全体の73%を占めている．厚生省の調査した108例のリソゾーム蓄積症の出生前診断例においては，Tay-Sachs病30例，Gaucher病20例，GM_1-gangliosidosis 12例，I-cell病10例で，これら4疾患の占める割合は約70%であり，われわれの成績とほぼ一致している．ところが，代謝性蓄積症患者400例中136例(34%)を占めるムコ多糖症について行われている出生前診断数は12例で，全体の11%と比較的少なく，これは本症の酵素診断に必要な基質が入手困難なことが多いことを反映しているとも思われた．このように，出生前診断の頻度が必ずしもその疾患の有病率を反映していないこともあるが，多くの場合，出生前診断の頻度は，その疾患の有病率を表現していると思われる．

A61. Prenatal Diagnosis of Morquio Syndrome: Tadao ORII, Kazuko SUKEGAWA, Hiroshi ICHIHASHI, Naomi ASANO, Fumiaki MOTOYOSHI (Dept. Pediatr., Gifu Univ., Gifu), **Eiichi TAMANAWA** (Nakagami Hosp., Okinawa) **and Masahiro YAMASATO** (Matsukawa Hosp., Okinawa)

The Morquio syndrome is a rare inherited disorder of mucopolysaccharide catabolism characterized clinically by retarded growth, dysostosis multiplex, corneal clouding, but normal intelligence. The patients excrete elevated amounts of keratan sulfate in their urine. We have investigated the fourth pregnancy of a woman whose first child had Morquio syndrome, diagnosed by the demonstration of *N*-acetylgalactosamine-6-sulfate sulfatase (GalNAc-6S sulfatase) deficiency in lymphocytes and in cultured fibroblasts. Amniocentesis was done in the 17th week of pregnancy. Confluent cultures of amniotic cells were assayed for activity of GalNAc-6S sulfatase. The specific activities showed the enzyme deficiency in the amniotic-fluid cells. But altered synthesis of mucopolysaccharides could

not be demonstrated. The pregnancy was terminated. Enzyme determinations in liver and brain of the aborted fetus confirmed the diagnosis. The residual activity in liver and brain was below 8% of that in control organs. Vacuolation and abnormal inclusions were seen by electron microscopy from liver, brain and other organs. The total amount of liver acid mucopolysaccharides was elevated 5.5-fold in the affected fetus compared with the control. A significant accumulation of keratan sulfate and chondroitin-6-sulfate was detected in the affected fetal liver. However, the total amount of brain acid mucopolysaccharides was within the normal range and a accumulation of keratan sulfate was not detected in the affected fetal brain.

A62. Genetic Amniocentesis for Prenatal Chromosome Detection in Japan (Follow-up Study of 1,517 Cases): Tadashi SUGAWA (Dept. Obst. Gynec., Osaka City Univ., Osaka), **Yoshiaki YAGAMI** (Dept. Obst. Gynec., Nagoya City Univ., Nagoya), **Takamichi SATO** (Dept. Obst. Gynec., Univ. Tokyo, Tokyo), **Yukio HISANAGA** (Dept. Obst. Gynec., Kyusyu Univ., Fukuoka), **Masami OZAKI** (Dept. Obst. Gynec., Osaka Univ., Osaka) **and Masahiko Matsumoto** (Dept. Obst., Gynec. Osaka City Perinat. C., Osaka)

From January 1971 to June 1981, 1,517 pregnancies in 1,365 women were investigated by mid-trimester amniocentesis for potential chromosomal aberration and X-linked hereditary disease, established by six laboratories participating in a collaborative study. The most common indication was the previous birth of a child with a chromosomal abnormality (67.5%), usually Down's syndrome. Fifty-seven tests (3.8%) gave an inclusive result. Chromosomal aberrations were disclosed in 69 fetuses out of 1,474 pregnancies in which the fetus was judged to be at risk of chromosomal aberration. The risk of recurrence of Down's syndrome was 1 : 100 when the mother's age was 34 years or less and 1 : 20 when the mother's age was 35 years or more. Twenty-three fetuses were found to be male from the tests for an X-linked disease. Nine tests (0.6%) gave an erroneous diagnosis including four errors regarding fetal sex and three errors regarding diagnostic dilemma of pseudomosaicism, which did not influence the outcome of the pregnancy. There were 58 artificial abortions as a result of the prenatal diagnosis. No significant maternal complications were observed. The total number of spontaneous abortions, intra-uterine fetal deaths and still-births following amniocenteses was 22 (1.5%), but amniocentesis-related risk of the feal loss after 1974 was considered less than 0.3%. The risk for physical abnormalities and developmental complications was not increased in children born after mid-trimester aminocentesis.

A63. 先天奇形の症状の変異について：木田盈四郎（帝京大・医・小児）. **Variations in Congenital Anomalies:** M. KIDA (Dept. Pediatr., Teikyo Univ., Tokyo)

常染色体性優性遺伝病を例にとると，Achondroplasia では患者の症状にあまり大きな個体差がないが，Holt-Oram 症候群では縦線型橈骨欠損に属する海豹肢症から母指欠損に至る様々な個体差があることはよく知られている．今回は，裂手裂足の自検例を対象に，症状の変異の問題を考えた．自検例は 77 例で，その内訳は一肢のみ 32 例，二肢 22 例，三肢 9 例，四肢のすべて 17 例であった．全体的にみると，右上肢 78 例，左上肢 69 例，右下肢 50 例，左下肢 48 例で，上肢が多いが左右差はない．同胞数は兄姉が 32 名，弟妹が 20 名でそのなかに同疾患のものはいなかった．親子例は，四肢にまたがった裂手裂足の母と息子の例と，父親が右 3 指列欠損でその娘に右 3 指列欠損，左 3・4 指列欠損，左 3 趾列欠損が認められた 2 例のみであった．裂手裂足は一般に常染色体性優性遺伝病と考えられているが，なかには胎芽病も混じっていると考えられる．症例を集めてさらに検討したい．

A64. Down Syndrome in Kanagawa, 1981–1983. Population Study by Kanagawa Monitoring Program (KAMP): Yoshikazu KUROKI and Yoshimitsu FUKUSHIMA (Div. Med Genet., Kanagawa Child. Med. Cent., Yokohama)

Kanagawa Birth Defects Monitoring Program (KAMP) has been in operation since October 1981 as the first population-based monitoring system in Japan. Down syndrome is one of the 48 marker malformations in KAMP. The number of total births including stillbirths during the 21-month period from October 1, 1981 to June 30, 1983 was 80,873. As 54 cases of Down syndrome were registered, the incidence was estimated to be 6.7 per 10,000 births. Though great fluctuation of incidences by month were observed, no statistically significant increase was noticed. The difficulty in accurate diagnosis of Down syndrome during the first week of life seemed to be the most plausible explanation of the low incidence of Down syndrome in KAMP. By record linkage study between KAMP records and Kanagawa Children's Medical Center records, the incidence of Down syndrome was expected to be 1 per 1,000 births. Maternal age-specific risks for Down syndrome in KAMP were studied. An increased risk was observed in the younger age groups and lower risk in the older age groups. It could not be determined, however, whether it was a real increase or an artifact. The possibility of an increased introduction of some environmental factors causing non-disjunction was discussed.

A65. 大阪府における先天異常モニタリングプログラム（第 4 報）：倉智敬一[1]，大浦敏明[2]，谷村　孝[3]，古山順一[4]，寺村定雄[5]，今川　誠[5]，福井雅夫[6]，竹村　喬[6,7]，林　昭[7]，張　知夫[7]，藤野俊夫[2]，荻田幸雄[8]，河村徹郎[7]，服部敏夫[7]，末原則幸[1]（[1]阪大・医・産婦人，[2]大阪市立小児保健センター，[3]近畿大・解剖，[4]兵庫医大・遺伝，[5]大阪府医師会，[6]大阪産婦人科医会，[7]大阪府母子センター，[8]大阪市母子センター）．**Monitoring Program for Birth Defects in Osaka (IV):** K. KURACHI *et al.* (Dept. Obst. Gynec., Osaka Univ., Osaka)

昭和 56 年 12 月より，大阪府において人口動態ベースの先天異常モニタリングで開始された．在胎満 24 週以降の死産児およびすべての生産児を対象とした．奇形は，生後 7 日目までに診断がついたものに限った．調査は全例報告式をとった．昭和 58 年 3 月までの 16 か月に，83,866 枚の調査票が回収された．全出産の母の平均年齢は 28.02 歳，35 歳以上の母の割合は 5.94%，死産児 0.68%，低体重児 5.77% であった．全奇形児の頻度は 1.08%，マーカー奇形をもつ児の頻度は 0.82 % であった．奇形発生ハイリスク因子（群）における奇形発生頻度をみると，双胎 2.08%，早産児 3.61%，低体重児 4.12%，また，早産でかつ児体重が −1.5 SD 以下の群では 13% であった．主なマーカー奇形の発生頻度（対出産 10,000）をみると，無脳 6.7，脊椎破裂 3.2，唇裂（口蓋裂合併を含む）13.9，口蓋裂 5.2，多指 7.5，多趾 4.3，合指 4.1，合趾 4.8，ダウン症候群（母 35 歳未満）5.6，同（母 35 歳以上）10.0 であった．

A66. 阪大病院における 10 年間の先天異常に関する調査（第 4 報）：末原則幸[1]，高井千秋[2]・武田佳子[2]・宇野敦子[2]・堀内登代子[2]，倉智敬一[1]（[1]阪大・医・産婦人，[2]附属助産婦学校）．**Analysis of Birth Defects in Newborns in Osaka University Hospital for the Past Ten Years (IV):** K. KURACHI *et al.* (Dept. Obst. Gynec., Osaka Univ., Osaka)

私たちは，阪大病院での先天異常児出産の実情を知るため，過去 10 年間の出産について調査を行った．対象は妊娠 24 週以降のすべての生産児および死産児で，奇形は生後 7 日目までに診断がついたものに限った．10 年間の全出産数は 4,730 で，奇形児数は 66，発生頻度 1.40% であった．主な奇形の発生頻度（対出産 10,000）は，耳介異常 25.4，口蓋裂 25.4，唇裂（口蓋裂合併を含む）10.6，無脳 12.7，多指 10.6，多趾 8.4，合指 4.2，合趾 8.5，ダウン症候群 2.1，であった．奇形発生の高率因子（群）をみると，母年齢 30～39 歳で 1.65%，2,500 g 未満の低体重児群，3.87%，早産児群，4.07% であった．また生死産別にみると，生産生存群 1.13% に対し，子宮内胎児死亡群 2.94%，分娩中死産群 33.33%，生後死亡群 88.89% と高率であった．母の合併症でみると，重症妊娠中毒症で，8.25% と高率であった．なお，奇形児のうち複合奇形をもつ児の割合は 28.7% であった．

B1. Blood Group Antigens A, B, and H were Determined by Means of Elution Test in Long-Term Cultured Human Lymphoblastoid Cell Lines: Tomoko HASHIMOTO, Yoshihiko NOJO, Kazue NAKAMURA, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med.) **and Hideyo YOKOYAMA** (Dept. Legal Med., Hyogo Coll. Med., Nishinomiya)

So far, no practical method for ABO blood group typing in long term cultured human cells has been described. Cells are usually cultured in medium supplemented with bovine serum. Therefore, we studied how, if it does, the serum interferes with the determination of the ABO-blood groups in cultured human cells, employing the elution test. All bovine sera tested contained antigen that reacted to human anti B-antibody (human B-like antigen). The group O human peripheral blood lymphocytes and EB-virus transformed lymphoblastoid cell lines which derived from the groups A, B, AB, and O donors had B-like antigen when these cells were incubated in RPMI 1640 supplemented with fetal bovine serum. These facts suggested that human B-like antigen present in the serum modify the antigenecity of human cells. Then, we used serum-free medium, HB 101 (HANA Media Inc.), which contained no antigens that reacted to human anti A- or anti B-antibodies. Lymphoblastoid cell lines maintained in RPMI 1640 with fetal bovine serum were washed with serum-free RPMI and resuspended in HB 101 medium. After 7–15 days' incubation in HB 101 medium, the proper ABO blood groups could be determined. The H-antigen could be also determined. Thus for proper ABO blood group typing, an antigen-free medium like serum-free HB 101 medium, seems to be indispensable.

B2. Assay for Blood Group Gene-Dependent Glycosyltransferases in Sera of A and B Subgroups by Conversion of Red Cells: Tamiko NAKAJIMA, Shin YAZAWA, Tadahisa KOGURE and Ken Furukawa (Dept. Legal Med., Sch. Med., Gunma Univ., Maebashi)

Standard incubation mixtures used for the enzymatic conversion of group O red cells to A or B cells are composed of serum 50 μl, nucleotide sugar 32.5 nmol, $MnCl_2$ 0.5 μmol, cacodylate buffer 34 μl and packed red cells 10 μl in total volume 120 μl. The mixtures were incubated for 14 hr at 37°C. The incorporation of α-*N*-acetylgalactosamine residues on group O saline red cells using group A_2 serum as enzyme source gives the cells a very poor A specificity which is difficult to observe by agglutination reaction with human anti-A. Group O papain treated red cells which incubated with UDP-*N*-acetylgalactosamine and serum from A subgroup individuals were found to agglutinate with rabbit anti-A immune serum. The agglutination titers of converted red cells by A_1, A_{int}, A_2 and A_3 sera were 1 : 16,384, 1 : 256–2,048, 1 : 64–256 and 1 : 2, respectively, with the rabbit anti-A. At the presence of UDP-galactose and serum of B_m or A_1B_3 individuals conversion of group

O red cells to B cell was detected by the agglutination with sheep anti-B immune serum. The conversion to group A active cells by the treatment of group O saline and papain treated red cells with UDP-*N*-acetylgalactosamine and group B serum indicate that group B serum has α-*N*-acetylgalactosaminyltransferase activity. The correct pH of variety of incubation mixtures was measured by micro-electrode pH meter and pH optimum of enzymes was determined. The A_1 transferase has a pH optimum at about pH 7.1, while the A_2 transferase has about pH 7.5. The results indicate that the weak A and B transferase activities can be detected using papain treated O red cells as acceptor and by the agglutination with immune antisera which contain IgG rich antibodies.

B3. Absorption and Elution of Lewis Blood Group Substances on Erythrocytes: Ken FURUKAWA, Tadahisa, KOGURE, Tamiko NAKAJIMA and Shin YAZAWA (Dept. Legal Med., Sch. Med., Gunma Univ., Maebashi)

Incidences of groups Le(a+b−), Le(a−b+) and Le(a−b−) among 1,053 nonpregnants were 22.8%, 70.8% and 6.4%, respectively, whereas those among 1,072 pregnant women were 0.3%, 54.6% and 25.1% respectively. The significant increase of incidence of group Le(a−b−) and the decrease of incidence of group Le(a−b+) among pregnant women were demonstrated. Lewis active glycolipid was eluted from erythrocytes after the incubation with pH 5.0 buffered saline. The supernatant of hemolyzed erythrocytes had stronger Lewis activity than the erythrocyte ghost. Lewis active glycolipid which was extracted from erythrocytes and plasma, and was fractionated by silicic acid column chromatography showed slower mobility on thin layer chromatography than those from erythrocytes obtained by the elution at pH 5.0 and from plasma glycolipid treated at pH 5.0. The Lewis active glycolipid extracted from erythrocytes and plasma may be complex of glycolipid and lipid or glycolipid and protein. The conversion of erythrocytes of group Le(a−b−) into groups Le(a+b−) and Le(a−b+) was demonstrated by incubation of erythrocytes with the Lewis active glycolipid, while the transformation did not occur by incubation of erythrocytes with intact plasma from group Le(a−b−) and Le(a−b+). Absorption of the Lewis active glycolipid on red cells was inhibited in the presence of lipoprotein of plasma.

B4. Polymorphism of Aldehyde Dehydrogenase: Immunological Detection of the Isozymes: Shoji HARADA, (Inst. Community Med., Univ. Tsukuba, Ibaraki) **Kaoru SAGISAKA** (Dept. Legal Med., Gifu Univ., Gufu) **and Toshiyuki KUDO** (Dept. Legal Med., Tohoku Univ., Sendai)

Two major isozymes (I and II) of aldehyde dehydrogenase (ALDH) were found in human liver. We have recently found that ALDH-I was missing in Japanese and other

Mongoloid population and that the deficiency of ALDH-I is responsible for alcohol sensitivity associated with facial flushing commonly observed in Oriental peoples (Harada *et al.*, 1980; 1981). We have now studied on the immonological method for detection of ALDH isozyme. ALDH-I and -II were isolated from human liver extract according to the method described by Hempel *et al.* (1982). Specific rabbit antisera against human ALDH-I and -II were prepared. The protein band possessing no enzymatic activity (ALDH-I deficiency) could be detected using antigen-antibody crossed immunoelectrophoresis. Thus, immunological technique used in the present study could be applied to identify the heterozygote phenotype of ALDH-I (normal/deficient). We have investigated the phenotypic distribution of ALDH-I in the districts of Gifu and Sendai. Gene frequency of Gifu and Sendai were 0.76 and 0.49, respectively. These result reflected the difference of alcohol consumption per year in two cities. Higher frequency of ALDH-I deficiency might give the influence to alcohol consumption due to biological sensitivity.

B5. Molecular Abnormality of an Inactive Aldehyde Dehydrogenase Variant Commonyl Found in Orientals: **Michiharu IKAWA** (Dept. Biochem. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo) **and Akira YOSHIDA** (Dept. Biochem. Genet., City of Hope Natl. Med. Cent., California)

Usual human livers contain two major aldehyde dehydrogenase isozymes, cytosolic $ALDH_1$ component and mitochondrial $ALDH_2$ component, while human livers with atypical phenotype have only $ALDH_1$ isozyme and are missing $ALDH_2$ isozyme. We previously demonstrated that atypical livers contained an enzymatically inactive but immunologically cross-reactive material (CRM), of which molecular weight and amino acid from composition were identical to the $ALDH_2$. The enzymatically active $ALDH_2$ obtained a usual liver and CRM obtained from an atypical liver were reduced, *S*-carboxymethylated, and digested by trypsin. Peptide mapping on a paper and separation of their digests by high performance reverse phase chromatography revealed that the $ALDH_2$ contained a peptide sequence of -Glu-Leu-Gly-Glu-Ala-Gly-Leu-Gln-Ala-Asn-Val-Gln-Val-Lys- and that Gln adjacent to Lys substituted by Lys in CRM. It is concluded that a point mutation occurred in the human $ALDH_2$ locus produced the Gln-Lys substitution and enzyme inactivation.

B6. Modified Protein Staining Method and a New Genetic Marker in Human Parotid Saliva: Kiyoshi MINAGUCHI and Kazuo SUZUKI (Dept. Forensic Odontol., Tokyo Dent. Coll., Chiba)

Genetic polymorphism of the Pmo (parotid modified staining) protein is detected by acid polyacrylamide gel electrophoresis and the modified staining method of Sung and Smithies. The protein migrates cathodally and somewhat faster than the Ps1 protein. The protein is either present (Pmo+) or absent (Pmo−) in parotid saliva. Family studies including 24 families and 57 offsprings were done to test the genetic hypothesis. The inheritance is assumed to be controlled by a dominant allele at an autosomal locus. For randomly collected salivas from a Japanese population, the gene frequencies are $Pmo^{+}=0.26$ and $Pmo^{-}=0.74$ ($N=202$). In tests of association with other salivary polymorphic phenotypes in randomly collected salivas from the Japanese, Pmo is strongly associated with Ps ($\chi^2=12.721$, $0.001>p$), and to a lesser degree with PmF ($\chi^2=5.985$, $0.02>p>0.01$), PmS ($\chi^2=5.519$, $0.02>p>0.01$), Pr ($\chi^2=5.921$, $0.10>p>0.05$), and Pa ($\chi^2=5.044$, $0.05>p>0.02$). The isoelectric point of the Pmo protein is in excess of 8.58 and the estimated molecular weight is 32,000. The biochemical characteristics and the results of the association studies suggest that the Pmo protein is one of the basic proline-rich proteins in human parotid saliva.

B7. Classification of α_2-HS-Glycoprotein (α_2HS) Types by Isoelectric Focusing: Kazuo UMETSU, Noriaki IKEDA, Seiichi KASHIMURA and Tsuneo SUZUKI (Dept. Forensic Med., Yamagata Univ. Sch. Med., Yamagata)

Polymorphism of the α_2-HS-glycoprotein (α_2HS) was investigated in Japanese. A sample of 1,100 sera from unrelated individuals from Northern Japan was analyzed by isoelectric focusing (pH 4–5) on polyacrylamide gels and immunofixation. Three common types, α_2HS 1-1, 2-1 and 2-2 were differentiated. The frequencies of the α_2HS alleles in our sample were found to be $\alpha_2HS^1=0.726$ and $\alpha_2HS^2=0.274$. Analysis of 34 parents with 53 children did not show any deviations from the expected mode of inheritance. From the result, α_2HS is found to be a useful genetic marker for human genetics, forensic science, and anthropologic research.

B8. Quantitative Studies in Genetic Types of Human Red Cell Esterase D (II): Itsuro NISHIGAKI, Tohru ITOH (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony) **and Nobuaki OGASAWARA** (Dept. Biochem., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

We recently pointed out that the mean values of red cell esterase D (EsD) activity varied significantly among the phenotypes. An approximate ratio 7 : 5 : 3 has been shown in individuals of EsD 1, EsD 2-1 and EsD 2 types, suggesting that the isoenzyme product of EsD^1 has catalytic activity 2.0–2.5 times higher than that of EsD^2. For further confirmations of such a quantitative variation, more blood samples of not only healthy individuals but also those with anemic state were investigated. Analysis in 804 healthy adults with common phenotypes showed almost the same distribution as previously reported (*Ann. Hum. Genet.* **47**: 187, 1983). While, a certain relationship between EsD activity and Hb content was observed in individuals with anemic state. In a range below 11.7 g/dl of Hb or 28 $\gamma\gamma$ of M.C.H. its relation was linear, with a correlation coefficient (r) -0.799. This factor due to hemoglobin was, however, eliminated by expressing EsD activity with units per cells. This suggests that activity values of EsD expressed with the unit per cells should be used in analyzing individuals with anemia. By means of concomitant isoelectric focusing studies, some individuals with a rare allele (EsD^7) were found. Their enzyme activity was shown to be extremely lower, comparing with those of common phenotypes ($EsD^1/EsD^7 = 3.5$; $EsD^2/EsD^7 = 1.5$). EsD activity in leukocytes and fibroblasts was additionally investigated. In both tissues there seemed to be not so large difference among phenotypes as seen in erythrocytes.

B9. The Distribution of Gc Subtypes in the Philippines: Keiichi OMOTO (Dept. Anthropol., Univ. Tokyo, Tokyo) **and Shogo MISAWA** (Dept. Legal. Med., Univ. Tsukuba, Ibaraki)

Gc subtypes were determined on a total of 1,646 serum samples obtained in the Philippines from 6 aboriginal Negrito populations (Aeta, Agta, Dumagat, Ati, Batak, Mamanwa), 2 slash-and-burn agriculturist populations of Mongoloid origin (Ifugao and Manobo) and 2 lowlander Filipino populations (Tagalog and Visayan). The frequenceis of alleles Gc*1F and Gc*1S vary considerably, but on the whole the frequency of the former is higher than that of the latter. In this respect, the Negrito populations resemble the Mongoloid populations of East Asia. The frequency of Gc*2 is relatively low in the Negrito populations particularly in the groups of northern Luzon. However, this frequency appears to be higher in the southern populations such as the Mamanwa (Negrito) and the Manobo (Mongoloid). The variant Gc*1N (Gc*1A3) which has been known to be the marker al-

lele of the Negrito group shows an interesting distribution: the highest frequency was found in the Aeta of west-central Luzon (0.1227 ± 0.0004) and it occurs also in the Batak of Palawan in considerable frequency (0.0294 ± 0.0017), suggesting the common origin of the two Negrito groups. Furthermore, it was found sporadically in the Ifugao of northern Luzon, Tagalog of Manila and Visayan of Negros Island, suggesting gene flow from the aboriginal Negrito groups. It was not found in the Mamanwa and other populations of the eastern part of the Philippines examined, indicating that this variant was confined in the Negrito groups of western part of the Philippines. This finding is consistent with the hypothesis that the Mamanwa belongs to the Protomalay racial groups rather than the Negritos.

B10. Cloning of Human mtDNA and Its Sequence Variation: Shinji HARIHARA, Masanori TAIRA, Katsuyuki YAGINUMA, Midori KOBAYASHI (Cancer Inst.), **Keiichi OMOTO** (Dept. Anthropol., Univ. Tokyo, Tokyo) **and Katsuro KOIKE** (Cancer Inst., Tokyo)

In order to study on variation of mitochondrial DNA (mtDNA), we purified mtDNA from placenta of 5 Japanese (H1, H2, H3, H4, H5) and cloned restriction fragments from each individual in pBR322.

Firstly, we cloned 5 different DNA fragments from one sample (H4). They are 4.1 kb and 3.0 kb *Eco*RI/*Sst*I fragments, 0.93 kb *Eco*RI/*Hind*III fragment, 1.1 kb *Eco*RI·C fragment and 3.4 kb *Hind*III/*Sst*I fragment. Within those, a 3.4 kb *Hind*III/*Sst*I fragment contains genes for cytochrome *c* oxidase subunit I, II, III, ATPase 6 and so on, so we cloned a corresponding mtDNA fragment from other three samples (H1, H3, H5) in a similar way to detect variation in this genome region. Then, we analyzed cleavage sites in the cloned fragments with 11 restriction enzymes (*Hha*I, *Bst*NI, *Acc*I, *Hinf*I, *Rsa*I, *Sau*3A, *Taq*I, *Alu*I, *Hph*I, *Mbo*II, *Hae*III). In addition to common cleavage sites, one extra *Hha*I site or *Alu*I was detected in the sample H4 or H1, respectively. These two cleavage sites are located in the cytochrome *c* oxidase subunit II and have not been reported previously. Based on the restriction maps, nucleotide sequence of this coding region of H1, H3 or H4 was determined. The obtained sequence is identical for different samples except that the point mutation created by A to G or T to C transition detected at the site 7,828 or 8,029 of Anderson's sequence in the H4 or H1 genome, respectively. According to the genetic code, these base substitution that distinguish *Hha*I or *Alu*I restriction maps occurs at the third base of codon. Therefore two different genotypes within Japanese is caused by a neutral or silent mutation.

B11. Hemoglobinopathy Survey in the Kobe District (II): Shunichi SHIMASAKI, Iwao IUCHI, Kazuo HIDAKA (Dept. Biochem., Kawasaki Med. Sch., Kurashiki) **and Wataru MIZUTA** (Kobe Municipal Cent. Hosp., Kobe)

Thirteen hemoglobin variants from a total of 36,320 specimens were detected for the past 3 years by the hemoglobinopathy survey in Kobe district. Three variants of them have been reported previously by us as Hb Ube 2 (α68 Asn→Asp), Hb Syracuse (β143 His→Pro) and Hb Suma (α11 Lys→Asn). In this meeting, we report four further variants which were identified as Hb Nunobiki (α141 Arg→Cys: 1 case), Hb G Coushatta (β22 Glu→Ala: 1 case) and Hb Ube 2 (α68 Asn→Asp: 2 cases). Hb Nunobiki which is a new variant is characterized by a high oxygen affinity and decreased alkaline Bohr effect, 2,3-DPG effect and Hill's constant n. The structural alteration in Hb Nunobiki seems to cause difficulty in the T structure formation, thereby causing a high oxygen affinity, because Arg residue at carboxy terminal of α chain is thought to play an important role for the formation of T structure. Hb G Coushatta showed the same functional characters (normal oxygen affinity, normal clinical findings and not particular hematological nature) as previously detected instances in Chinese, Koreans, North American Indians and Japanese. Hb Ube 2 has been thought to be sporadically distributed only among Japanese. One of three Hb Ube 2 carriers detected in this study, however, was a Korean. This is the first case detected in Korean. Frequency of abnormal hemoglobin detected in Kobe district was 1/2,800 indicating similar values to those of other districts in Japan (1/2,700–1/4,000).

B12. Frequency of Thermostability Variants of Erythrocyte Enzymes: Chiyoko SATOH, Hideo OHMINE, Akiko MIURA, Hiroko ARAKAWA, Chiemi UENO, Mikio FUJITA and Kazuaki GORIKI (RERF, Hiroshima)

For evaluation of the genetic effects of A-bomb radiation at the protein level, the proteins in blood of children born to proximally exposed A-bomb survivors and those born to distally exposed survivors (controls) were examined for variants which show abnormality in mobility of starch gel electrophoresis, enzyme activity or thermostability. When variants were detected, the tests were conducted on the parents to ascertain whether the variants were due to fresh mutation or was inherited. This report will describe the thermostability of seven erythrocyte enzymes in approximately 1,000 children of Hiroshima. A 1 : 20 diluted hemolysate was prepared according to the method recommended by ICSH using red cells preserved in liquid nitrogen and heated at 50°C for 20 and 30 min (AK1), at 52.5°C for 20 and 30 min (PGK, GPI, PK, 6PGD), and at 60°C for 10 and 20 min (GOT1, LDH). The residual activity after heating was expressed by percentage against activity

in non-treated hemolysate, and the mean value and standard deviation (SD) of residual activity of each enzyme were calculated for each of the heating condition. The number of tests varied according to the enzyme from a minimum of 399 (AK1) up to a maximum of 1,383 (GPI) for a total of 5,930. Nine individuals showed the genetic abnormality in thermostability confirmed by family studies with the initial and the newly obtained samples: those with a residual activity of 2.5 SD less than the mean value were 3 out of 1,383 cases in GPI, 3 out of 461 cases in PGK, 1 out of 1,037 cases in 6PGD and 1 out of 399 cases in AKI, and 1 out of 627 cases in LDH showed residual activity of 2.5 SD more than the mean value. Frequency of variants varied from enzyme to enzyme with a mean of 2.4/1,000, which was the same as that of low activity variants (2.4/1,000) and electrophoretic variants (2.3/1,000).

B13. Isolation and Partial Characterization of the Three Major Homozygous Group-Specific Components from Human Plasma: Toyohito HARA, Shigenori ITO and Hideo MATSUMOTO (Dept. Legal Med., Osaka Med. Sch., Takatsuki)

Group-specific component (Gc) homozygous protein was isolated with good yield and the separation of the two components (anodal and cathodal) of Gc1F and of Gc1S protein was achieved. The isolation procedures were as follows: ammonium sulfate fractionation, Blue Sepharose CL-6B affinity chromatography, first DEAE-Sepharose CL-6B chromatography (pH 8.8), second DEAE-Sepharose CL-6B chromatography (pH 6.2), and gel filtration on Sephadex G-75 superfine. The separation of the anodal and cathodal component of Gc1F and of Gc1S was achieved in the second step of DEAE-Sepharose CL-6B chromatography using a narrow column, instead of column isoelectric focusing technique which has been used previously. Each purified protein was shown to consist of a single polypeptide chain of molecular weight 49,000 and to have leucine as the amino-terminus. The amino acid composition of these Gc proteins were analyzed and distinct differences were found among three homozygous Gc types, Gc1F, Gc1S and Gc2.

B14. Amino Acid Sequence and Evolution of Human Immunoglobulin D: Tomotaka SHINODA, Fuyuki KAMETANI and Tatsunori TAKAYASU (Dept. Chem., Tokyo Metrop. Univ., Tokyo)

The complete covalent structure of a monoclonal human immunoglobulin D has been determined by sequence analyses of the peptides and fragments obtained either by digestion with trypsin, chymotrypsin, pepsin and V8 protease, or by cleavage with cyanogen bromide of the delta chain, Fd and Fc fragments which had been prepared by limited pro-

teolysis of the whole IgD preparation NIG-65. The light chain type was lambda and it consisted of 216 residues with two extra amino acid residues in between 27–28. The V-region characteristic was found to be VλVI subgroup, which was a new lambda chain subgroup. On the other hand the sequence of the C-region was essentially the same as that of protein Ha which had been determined by the author in 1970. The delta heavy chain consisting of 507 residues had the NH_2-terminal sequence of Ala-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Ala-Leu-Val-, and ended with a methionine residue. The V-region covering 120 residues has the VH III subgroup specificity. The hinge region consisting of 63 residues, which is about three times as long as those of the alpha and gamma, has all the galactosamine-containing carbohydrates of the delta chain. We have demonstrated three sequence rules with regard to the carbohydrate binding: one of these is Val-Pro-Thr-, and the others being Ala-X-Ala-Thr-Thr- and Ala-X-Ala-Ser-Ser-, where X may be any amino acid including proline. We therefore designate the former as triplet rule, and the latter as quintet rule. On the other hand, all the glucosamine-containing carbohydrates were found in the Fc region. They located at positions Asn-348, Asn-440, and Asn-491, respectively. The Fc region consisted of 226 amino acid residues and had the NH_2-terminal sequence of Thr-Pro-Glu-Cys-Pro-Ser-His-Thr-, and the C-terminal of methionine. Although the Fc region of the delta chain has the common framework structure of immunoglobulin, its sequence has many individual characteristics when its two domains are compared separately with the counterpart domain of other heavy chains. Such comparison has shown the two domains of the Fc fragment of the delta chain should be placed in an independent phylogenic branch, and that in the course of evolution, the Cδ-gene diverged early from other immunoglobulin gene families. It has also speculated that the primodial domain gene may have coded for a unit about the size of a half domain. Based on this observation together with the sequence comparisons, a possible genetic mechanism is proposed for the origin and evolution of the genes for immunoglobulin domains.

B15. A New Immunoglobulin Marker of Human Lambda Light Chain: Tomotaka SHINODA, Fuyuki KAMETANI, Kazunori YOSHIMURA (Dept. Chem., Tokyo Metrop. Univ., Tokyo) **and Takashi ISOBE** (Dept. Med., Kobe Univ., Kobe)

During the course of sequence analyses on 16 human lambda chains, one specimen (NIG-68) was found to have amino acid substitutions which have not previously been reported for the constant region of the lambda light chain. The specimen, purified from the urine of a patient with multiple myeloma associated with Fanconi syndrome, was digested with trypsin and V8 protease after the complete reduction and aminoethylation. The peptides following the purification by reverse phase HPLC were sequenced by com-

bination of manual Edman degradation and direct identification of PTH-amino acids by HPLC in the conventional buffer system. NIG-68 consisted of 212 residues with the NH_2-terminal sequence of Tyr-Asp-Leu-Thr-Gln-Ala-Pro-Ser-Leu-Ser- which is characteristic of the VλIV subgroup. It had rare amino acid replacements in the constant region: an arginine instead of lysine at position 130 and a leucine for glutamine at position 195. Since the variation is unreported one for human lambda light chain, it is tentatively designated as "Is" marker. Although the sequence for the marker is established, its biological significances are not understood at present. Whether or not it is allotype is also remained to be clarified.

B16. Interspecies Cross-Reactivity of Monoclonal Anti-MB1 Antibodies: Kazumasa OGASAWARA, Masanori KASAHARA, Tsuguyo OKUYAMA, Hiroshi KUNIKANE, Yuichiro FUKAZAWA, Akemi WAKISAKA, Yuko KIKUCHI and Miki AIZAWA (Dept. Pathol., Hokkaido Univ. Sch. Med., Sapporo)

The major histocompatibility complex (MHC) is a cluster of loci occupying a single chromosome area, the products of which are involved in various important immunological phenomena. One of the most interesting characteristics of MHC is that antibodies raised against monomorphic or polymorphic determinants of class I and class II antigens show alloreactive cross-reaction across the species barrier. However, it has not been reported that antibodies raised against polymorphic determinants of human class II antigens detect polymorphic determinants of class II antigens in other species. The present study demonstrates that the four monoclonal antibodies HU-10, HU-11, HU-32 and HU-33 specific for two distinct polymorphic determinants of human class II antigens cross-react with rat B-cells carrying a polymorphic determinant Ba-2.6. To our knowledge, this is the first report showing that xenoimmune antibodies raised against polymorphic determinants of human class II antigens are able to recognize polymorphic determinants of class II antigens in other species. This phenomenon demonstrating the sharing of polymorphic determinants of class II antigens between humans and rats appears to be explained most easily by the single locus-old alleles hypothesis proposed by Klein and co-workers.

B17. Study of Genetic Variation among the Japanese (in Hiroshima and Nagasaki) Using Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE). I. Differences of Genetic Variation in Plasma Proteins between the Populations: Norio TAKAHASHI, Jun-ichi ASAKAWA, Mikio FUJITA, Chiyoko SATOH (RERF, Hiroshima) **and Ryuji HAZAMA** (RERF, Nagasaki)

Genetic variations of plasma proteins in the Japanese (Hiroshima, Nagasaki) were studied using 2D-PAGE. Plasma samples were obtained from 98 families of the subjects in the

ongoing biochemical genetics study of Radiation Effects Research Foundation. Electrophoresis was performed by a modification of the method of Anderson *et al.* Peptides were first stained by Coomassie blue and then by the silver staining method of Sammons *et al.* Of the numerous peptides detected, 23 (12 from Coomassie blue staining and 11 from silver staining) were scored for genetic variations. Genetic variants were detected for 8 peptides (6 from Coomassie blue staining and 2 from silver staining). This paper describes genetic variants of apolipoprotein E (Apo E) and D-05 (an unidentified peptide) detected by silver staining. For D-05, variants with higher isoelectric point and those with higher molecular weight than the normal type were found in the Japanese population, but the latter have not been observed in both Amerindians[1] and Caucasians[2]. For Apo E, three isopeptides (Apo E II, Apo E III and Apo E IV) have been found in the Japanese as well as in the Caucasian population, while Apo E II has not been observed in the Amerindian population. These results suggest that variant types and their frequency differ among populations.

1) Asakawa, J. *et al.*: *Electrophoresis*, in press.
2) Neel, J.V. *et al.*: *Methods and Applications of Two-Dimensional Gel Electrophoresis of Proteins*, Cellis, J.E., ed., Academic Press, New York, in press.

B18. Study of Genetic Variation among the Japanese (in Hiroshima and Nagasaki) Using Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE). II. Genetic Variants in Plasma Protein Detected by Coomassie Blue Staining: Junichi ASAKAWA, Norio TAKAHASHI, Mikio FUJITA, Chiyoko SATOH (RERF, Hiroshima) **and Ryuji HAZAMA** (RERF, Nagasaki)

Using 2D-PAGE with Coomassie blue staining, a total of 12 peptides were evaluated for genetic variation and variants were detected in 6 peptides, that is, hemopexin (Hpx), transferrin (Tf), Gc-globulin (Gc), prealbumin, and unidentified peptide B-03 and C-11. Family studies confirmed that all variants are inherited traits. Hpx is polymorphic in the Japanese. The variant has same molecular weight, but anodal migration results in doublet spots. Tf B and Tf D variants were detected in Tf and the variant with a higher isoelectric point (pI) was detected in prealbumin. The Gc variant has higher molecular weight than Gc-1, but is not separable from it by focusing. All of these variants were identified by commercially available antiserum. The B-03 variant with a higher pI occur in lower frequency than found earlier in Caucasoides. We identified C-11 as apolipoprotein A-IV (apo A-IV) by rabbit antiserum against human apo A-IV (This antiserum was kind gift from Dr. S. Koga, Kyushu Univ.). Genetic variants of apo A-IV with a higher pI were reported by Tracy *et al.*[1] and Neel *et al.* using 2D-PAGE, and by Utermann *et al.*[2] and Menzel *et al.*[3] using both 1D and 2D-PAGE. On the other hand, we found 3 variants

of apo A-IV with a lower pI in 98 Japanese, and Asakawa *et al.* reported 7 variants with a lower pI in 105 Amerindian.

1) Tracy, R.P. *et al.* 1982. *Clin. Chem.* **28**: 890-899.
2) Utermann, G. *et al.* 1982. *J. Biol. Chem.* **257**: 501-507.
3) Menzel, H.J. *et al.* 1982. *Hum. Genet.* **62**: 349-352.

B19. How Many Traits Are Necessary for Determining the Genetic Distance: Tsutomu MIYASHITA and Koji OHKURA (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The number and the kind of genetic markers are very important in determining genetic distance between populations. To reveal minimal number of markers and adequate sort of makers, we used such four populations as Japanese, Korean, Iranian and English. When the distances between each two populations are measured by the gene frequencies on 14 markers (ABO, MN, Ss, Rh-C c, E e, P, Duffy, Diego, Kidd, Kell, PGM, Hp, Gc and Catalase), the distances are estimated as follows: Iran-England (0.118), Iran-Japan (0.270), Iran-Korea (0.307), England-Japan (0.307), England-Korea (0.334). If one marker is omitted from these 14 markers, in any case the value of distances and alignment order are not changed. However, if the number of markers is reduced to 9 from 14, the alignment orders are almost same, but the distances become different in each combination of markers. Although the alignment orders are not much changed, the deviation of distances between the populations become larger according to reduction of number of markers. From these observations, it is concluded that the number of markers should be more than 9, and at least ABO, MN, Ss, and all subtypes of Rh should be included. The genetic distance was analysed by the method of Cavalli-Sforza and Edwards. For the data on gene frequency in Iran, Japan and Korea ours were used (1960s–1980) and for those in England, we used the tables of Mourant and Kopec (1976).

B20. 日本における先天性代謝異常の地理的分布：安田徳一（放医研・遺伝）. **Geographical Variations in Inborn Errors of Metabolism in Japan:** Norikazu YASUDA (Div. Genet., Natl. Inst. Radiological Sci., Chiba)

昭和 52 年度より開始された先天性代謝異常等検査実施状況（厚生省）の資料にもとづき，わが国における常染色体劣性遺伝病の地理的分布を検討した．検査が軌道にのったと考えられる昭和 54 年度以降の 3 か年間の出生総数は 4,708,874 で，その 94% がヒスチジン血症，フェニールケトン尿症，ガラクトース血症，ホモシスチン尿症，その他の検査を受け，それぞれ 621, 72, 61, 31 名の患者が発見された．これは 100 万出生当たり，ヒスチジン血症で 140，フェニールケトン尿症で 16，ガラクトース血症で 14，ホモシスチン尿症で 7 に相当する．都道府県別の発生率を検討したところ，ヒ

スチジン血症に関しては，瀬戸内海をはさんで中国，四国地方に高く，東北地方で低い傾向が認められた．ヒスチジン血症が日本全体で一様に発生しているという仮説は統計的に棄却された．その他の異常についても同様な傾向が認められた．劣性遺伝病の地理的変異の要因として近親婚が考えられるが，ほぼ 30 年前（昭和 25 年ころ）の全国の近親婚率の資料がないため明らかではないが，昭和 47 年の全国調査資料（今泉ら，1975）により検討したところ相関はみられなかった．遺伝子頻度の機会的変動の可能性がヒスチジン血症で示唆されたが，他の異常では頻度も低く不明である．淘汰の地域差については資料がなく手掛かりは得られなかった．

B21. Genetics and Prevention Program of Duchenne Muscular Dystrophy (II). Situations in the National Hospitals for Muscle Diseases: Kiyotaro KONDO, Keiko FUJIKI and Masako TANIMURA (Dept. Neurol., Tokyo Metrop. Inst. Neurosci., Tokyo)

A total of 1,000 beds are provided for care, rehabilitation and schooling for the patients with Duchenne dystrophy (DMD) in 25 national hospitals throuout Japan. This excellent care program does not include genetic counseling, however. In the previous report, we noted that DMD is inherited X-linked recessively in more than 95% of the patient, the rest being autosomal recessive. Incidence rate was 21.7×10^{-5} among newborn males, and mutation rate was 6.3×10^{-5} per locus per generation with no difference between sperms and ova. A survey disclosed that 17.0% and 38.2% of the fathers and mothers, 2.8% and 3.1% of the normal brothers and sisters of patients, respectively, were examined for carrier state. 57.6% of the probable carriers were identified by the serum creatine phosphokinase activities (CPK). Among 55 expert physicians in these hospitals, 1) 65.3% explained genetic mechanisms of DMD, 2) their majority measured CPK only when opportunity was available, abnormal values were not necessarily reported to the family members, 3) 43.6% experienced genetic counseling, mostly retrospective, 4) 16.3% discussed male abortion, but carried out in one case only. Unlike to the care programs, those for genetic counseling and prevention are yet to be explored.

B22. インスリン依存型糖尿病の発症年齢分布からみた遺伝的異質性：古庄敏行（杏林大・保健・疫学），**金沢康徳**（東大・医・3 内），**丸山　博**（東女医大・小児），**吉丸博志**（杏林大・保健・疫学）．**Studies on Genetic Heterogeneity of Insulin Dependent Diabetes Mellitus on the Frequency Distribution of Age of Onset:** T. FURUSHO (Dept. Epidemiol., Sch. Health Sci., Kyorin Univ.), Y. KANAZAWA (3rd Dept. Int. Med., Fac. Med. Univ. Tokyo, Tokyo), H. MARUYAMA (Dept. Pediatr., Tokyo Women's Med. Coll., Tokyo) and H. YOSHIMARU (Dept. Epidemiol., Sch. Health Sci., Kyorin Univ., Tokyo)

Bell (1935, 1939) の発症年齢と遺伝様式との関係の研究以来，多くの疾患において両者の関係が報告され，発症年齢分布は従来同一と考えられていた疾患の遺伝的異質性の分析に有効な要因の一つと

されている．われわれはインスリン依存型糖尿病 (IDDM) の発症年齢分布資料を用い，複合正規分布を仮定して，遺伝的異質性の分析を試みた．A) 小坂ら (1978) の IDDM の発症年齢分布資料では，単一の正規分布の仮説にも複合正規分布の仮説にも適合しないが，統計的有意水準からみると，複合正規分布を仮定するほうが確率は大きい．若年発症群と成人発症群に異質性が存在する可能性は残されている．B) 古庄ら（投稿中）の若年発症 IDDM の資料，および金沢ら（本研究）の IDDM 資料のうちの若年発症群の資料では，いずれも，単一の正規分布の仮説には適合せず，複合正規分布の仮説にはよく適合した．分類の判定基準値は，古庄らの資料では男女合計で 5.8 歳，金沢らの資料では，男で 7.8 歳，女で 7.3 歳，合計で 7.6 歳と推定された．このことは，若年発症群の中にも異質性があることを示唆している．

B23. Genetic Analysis of Keratoconus: U. TANABE, K. FUJIKI, A. KANAI, A. OGAWA, K. NAKANO and A. NAKAJIMA (Dept. Ophthalmol., Juntendo Univ., Tokyo)

The prevalence of keratoconus in Japan is unknown. In order to estimate it, we surveyed the number of keratoconus patients in Juntendo University and other 75 institutions in Japan. Of the 2,601 patients, 1,813 are male and 788 are female. 1,618 patients were seen in Juntendo University from 1962 to June, 1983 and the others at 53 institutions in 1982. The percentage of replies from institutions was 69%. About 25% of the patients were born during the year of 1952 to 1956. There were 485 males and 171 females. We adjusted the number from 485 and 171 to 550 and 192, considering the 31% of non-reply. Then the prevalence was calculated as 12.8×10^{-5} in male and 4.5×10^{-5} in females. Two families with the affected sibs of only male were found among 164 families of male propositi. Using these family data and the prevalence of male, heritability approximated $h^2=79\pm13\%$ by Falconer's method 1. According to Edwards' model, the observed value fit in hypothesis of polygene. On Newcomb's test, the observed value was plotted on the line of multifactorial inheritance. There were no significant effects of the birth order and the maternal age on birth of keratoconus patients.

B24. Statistical Analysis on Mortality Rates for Amyotrophic Lateral Sclerosis in Japan: Yoko IMAIZUMI (Inst. Population Prob., Ministry of Health and Welfare, Tokyo)

The death rate of amyotrophic lateral sclerosis (ALS) was analyzed using Japanese vital statistics for the period 1968–1978. The total number of male deaths by ALS was 142 in 1968 and 266 in 1978. The corresponding death rates per 100,000 population were 0.285 and 0.472, respectively. Similarly, the total number of female deaths by ALS was 73 in 1968 and 177 in 1978. The corresponding death rates per 100,000 population were

0.142 and 0.304 respectively. Therefore, the ALS death rate was statistically significantly increased with the year for both sexes. Average annual age-adjusted ALS death rates by age, sex and marital status were computed using age-specific ALS death rates in each marital status during the period 1969–1978. The lowest age-adjusted death rate was seen in the married group for both sexes, whereas the highest rate for males was seen in the widowed group and for females in the single group. Therefore, there were remarkable differences in the ALS death rates for each sex among the four marital categories. The geographical variations in the ALS death rate indicated that the highest death rate per 100,000 population is in Wakayama Prefecture for both sexes (1.526 for males and 0.678 for females). These rates were 2.8 times for males and 2.2 times for females as high as the overall ALS death rate. The mean age at death for ALS gradually increased year by year for both sexes, but the age was 12 years shorter for males and 17 years shorter for females than that of the general population.

B25. An Epidemiologic Study on Familial Predisposition to Large Bowel Cancer. Second Report: Motoi MURATA (Div. Epidemiol., Chiba Cancer Cent., Chiba) **and Takashi TAKAHASHI** (Dept. Surg., Cancer Inst. Hosp., Tokyo)

This study was aimed to clarify clinico-epidemiological characteristics of a large bowel cancer which might be genetically predisposed but unrelated to familial polyposis coli. Materials were hospital records of 937 male and 689 female patients who were admitted to the Cancer Institute Hospital, Tokyo, in 1946–1979. As was introduced in the previous report (the 27th annual meeting of JSHG), age at surgery, site and/or multiplicity of tumor, and polyp manifestation were compared between those cases with and without a positive family history of large bowel cancer. Familial patients (about 6% of total patients) were distinguished with 1) predominancy of multiple tumor, double primary cancer in other organs or proximal colon cancer, 2) younger age at onset, and 3) slightly higher frequency of polyp manifestation, in addition to 4) the positive family history. Although a positive family history may not by itself imply a genetic predisposition, multiple appearance of cancer within a family is very likely to indicate that it should be caused by some genetic factors. Thus, further comparison was proceeded between those cases with two or more relatives with the large bowel cancer (multiplex familial patients) and the others. Such families were detected in 16 patients (about 1% of total), which hold about 18% of all familial patients. This proportion is elevated rather constantly to 25–30% when other conditions such as age less than 50 of onset, multiplicity, double primary cancer and/or proximity of tumor site are included. Polyp manifestation showed negative effect on this proportion. Thus those characteristics mentioned above for a familial patient could also be applied as it is to the hereditary case.

B26. Serum Requirements of Skin Fibroblasts from Patients with Tuberous Sclerosis: Kousaku OHNO and Kenzo TAKESHITA (Div. Child Neurol., Inst. Neurol. Sci., Tottori Univ. Sch. Med., Yonago)

Serum requirements have been studied in culture fibroblasts from patients with tuberous sclerosis (TS). The parameters consisted of 1) saturation density in 10% fetal bovine serum (FBS), 2) that in 2% FBS, 3) ratio of saturation density in 2% FBS to that in 2% FBS, and 4) colony-forming efficiency (CFE) in 2% FBS. Cell strains from patients with TS (6 strains), Recklinghausen's neurofibromatosis (NF) (4 strains), adenomatosis of the colon and rectum (ACR) (2 strains) and a basal cell nevus syndrome (BCN) and from apparently normal individuals (9 strains) were included for the present study. Skin fibroblasts from a patients with Lesch-Nyhan syndrome were transformed by SV40 (SVLN) and also used for the study. The average of 3–4 independent experiments for each strain was calculated in each parameter. Comparing 6 TS strains, 4 NF strains, or 2 ACR strains with 9 normal strains using *t*-test, there were no difference in these four parameters. However the SVLN strain had significantly lower serum requirements in all parameters compared to the average values of normal control strains, and a TS strain, a NF strain and a BCN strain had lower serum requirements in terms of saturation density ratio (2%/10%) and CFE in 2% FBS. Our results show that fibroblasts from patients with TS, NF and ACR can not distinguish from those from normal donors by these parameters. Three strains from patients with different disorders showed slightly lower serum requirements. The reasons remain obscure, however, the ability to proliferate in low serum of these strains is obviously less than that of the SV40 transformed strain.

B27. Comparative Analysis of Methotrexate-Synchronized (MTX) and Non-Synchronized (Cont) Culture Techniques in Bone Marrows of Patients with Hematologic Disease: Takafumi TOMIYASU, Michiko OKADA, Kura KUBOTA and Yoshiko NOMURA (Chromosome Lab., Shiseikai Dai-ni Hosp., Tokyo)

MTX and cont culture techniques were compared on the proportions of chromosomally abnormal and normal cells (or cells with Ph^1 translocation as sole chromosomal abnormality and cells with additional abnormality) in bone marrows of 9 patients with hematologic disease. Three patients with acute non-lymphocytic leukemia (ANLL) demonstrated the significant differences in the cell proportions between the 2 cultures. In the 1-day cultures from the 1st patient, abnormal cells were less frequently observed in the MTX culture. In the 2-, or 3-day cultures, the MTX culture yielded a higher proportion of abnormal cells than in the cont culture in the 2nd patient, or disclosed in the 3rd patient a clonal chromosomal abnormality that was not detected in the cont culture. The three

patients had leukemic blasts below 50% in their bone marrows. We previously experienced 3 cases (1 published and the others in press) showing the findings similar to those of the present 1st patient, and they also had blasts below 50% in their marrows. Our findings obtained from the present and the previous data suggest that the MTX culture technique with 1-day cultivation is efficient to detect both normal and abnormal metaphases, especially in patients with blasts below 50% in their bone marrows.

B28. Chromosome Translocations in the Genesis of Leukemias: The Importance of Both 15 and 17 Chromosomes in Acute Promyelocytic Leukemia: Takaaki ISHIHARA and Masako MINAMIHISAMATSU (Dept. Radiatr. Hazards, Natl. Inst. Radiol. Sci., Chiba)

With regard to some specific chromosome translocations such as t(9;22), t(8;21), t(15;17), and t(8;14) which characterize respective leukemias and malignant lymphomas, it seems to be an important problem to solve whether both chromosomes involved in each translocation are vital to the genesis of the respective disease or either one of them may bring about the effect, and whether a translocation involving only specific break points produces a specific effect or not. We would like to report here our results of the chromosome analysis in acute promyelocytic leukemia (APL) which may shed some light on the problem. Whereas the translocation t(15;17) has been known to be characterizing APL, a case of suspected APL with a variant translocation involving No. 15 but not No. 17, t(9;15)(q13;q22), was found in our laboratory. On the other hand, two cases of APL with variant translocations involving No. 17 but not No. 15 were reported by Yamada, *et al.* (*Cancer Genet. Cytogenet.*, 9, 1983). The presence of these two opposite types of variant translocations seems to indicate that genetic factors related to the genesis of APL may lie in both No. 15 and No. 17. Of course, the optimal condition for the pathogenesis of APL should be the combination of the two as indicated by the fact that the 15;17 translocation had been found in the majority, but variant translocations involving either No. 15 or No. 17 alone may possibly lead to the development of the disease.

B29. Cytogenetic Studies in Myelodysplastic Syndrome: Masafumi TANIWAKI, Kazuhiro NISHIDA, Taira MAEKAWA, Junichi EDAGAWA, Yoshiaki SONODA, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (Dept. Med., Kyoto Pref. Univ. Med., Kyoto)

The chromosomes of bone marrow cells from 24 patients with myelodysplastic syndrome (MDS) were identified with the various banding techniques including new banding methods using base specific antibiotics and fluorochrome, such as actinomycin D, distamycin A,

and DAPI (4'-6-diaminido-2-phenylindole). MDS was subclassified into the 6 categolies according to the FAB classification: RA (3 cases), RA with ring sideroblasts (1 case), RAEB (9 cases), RAEB in transformation (6 cases), CMML (1 case), and the unclassified cases (3 case). Seventeen cases of them (71%) showed various chromosome abnormalities. It became evident that the structural rearrangements frequently involved the short arm of No. 2, the long arm of No. 5 and 6, and the whole arm of No. 1. No cases with the 8/21 an 15/17 translocation, commonly known as the specific chromosome abnormalities in acute nonlymphocytic leukemia, were found in our series. The segment q25–26 of chromosome 6 was involved in all cases with 6q abnormality. In recent years, 5q− and 7q− have been suggested as the specific chromosome abnormalities for preleukemic states. Our studies presented here suggested that the abnormalities of 2p and 6q were also probably specific for MDS. To confirm our hypothesis, however, further cytogenetic studies in MDS and/or preleukemic states will be recommended.

B30. The Heritability of Liability to Cancer, Estimated by Case-Control Studies: **Naoyuki OKAMOTO and Mineo WATANABE** (Dept. Hyg., Tottori Univ. Sch. Med., Yonago)

In Yonago City, Tottori Prefecture, Japan, 551 death certificates of which the diagnosis were epitherial malignant neoplasm (International Classification of Disease (ICD), 8th revision, Code No. 151, 152, 153, 154, 162), were examined for six years from January 1, 1974 to December 31, 1979. 174 patients of them were selected as case propositi and 174 intact inhabitants who matched with sex, age and residence area were set up as control propositi. Then the 174 matched pair samples were interviewed and analyzed for cancer onset of relatives. The total incidence in parents, sibs and offsprings were 7.1% (100/1,407) for relatives of cases and 3.2% (45/1,416) for relatives of controls. The value of heritability (h^2), with its standard error (SE), has been estimated as $33\pm7\%$ using the Method 2 of Falconer (1965). As it has been observed usually that the incidence of malignant neoplasm has been varied by sex, so each sexes of propositi and relatives were treated separately. And the four estimated values of '$h^2\pm$SE (%)' are as follows: $29\pm12\%$ for male-male, $20\pm13\%$ for male-female, $36\pm14\%$ for female-male and $57\pm14\%$ for female-female. The values of '$h^2\pm$SE (%)' among relatives of case propositi with cancer of stomach, intestines and lung were $31\pm8\%$, $46\pm13\%$ and $31\pm10\%$, respectively. It is shown in this study that the value of heritability among relatives of the female propositi is higher than those of the male and the value of heritability among relatives of propositi with malignant neoplasm of intestines is also higher than the other samples.

B31. 散発性網膜芽細胞腫の患児出生にみられる季節変動の検討：松永　英（国立遺伝研），**箕田健生**（都養育院病院・眼科）．**Sporadic Retinoblastoma: Lack of Seasonal Variation in the Births of Patients:** Ei MATSUNAGA (Natl. Inst. Genet., Mishima) and Kensei MINODA (Tokyo Metrop. Yoiku-In Hosp., Tokyo)

網膜芽細胞腫 (R) の 95% は散発性に発生し，両眼性 R はすべて優性に遺伝するが，片眼性 R の大多数は非遺伝性である．非遺伝性 R を誘発する環境因子としては，胎児期から出生前後にかけて作用する向神経性の化学変異原やウイルスが疑われる．例えばニトロソ化合物は，動物実験（経胎盤ないし新生仔期の投与）によって高率に中枢神経系腫瘍を誘発し (Druckrey ら，1966)，ヒト adenovirus 12 は，動物の眼球内に注射すると R 様腫瘍を生じ (Mukai ら，1980)，試験管内ではヒト胎芽の網膜細胞を形質転換する (Byrd ら，1982) ことが知られている．ニトロソ化合物の体内産生や adnovirus 12 の活動は，季節変動を受ける可能性が高い．散発性 R 患児の出生月変動を調べた報告はこれまでにいくつかあるが，適切な分析をするにはどれも症例数が少なすぎるものであった．今回われわれは，網膜芽細胞腫全国登録資料に基づいて，1965～1981 年に生まれた 981 例（片眼例 675，両眼例 306）の散発性症例の出生月変動を分析した．対照は，人口動態統計を患児出生年によって規準化したものを用いた．その結果，片眼例・両眼例の出生月には有意な季節変動のないことが判明した．

B32. A Study of the Genetic Effects of Occupational Exposure to Mustard Gas (II): Hideo OHMINE, Mikio FUJITA, Kazuaki GORIKI, Junichi ASAKAWA, Chiyoko SATOH (RERF, Hiroshima), **Michio YAMAKIDO and Yukio NISHIMOTO** (Sec. Dept. Intern. Med., Hiroshima Univ., Hiroshima)

It has already been reported that former workers of the Ohkunojima mustard gas factory in Takehara City, Hiroshima Prefecture, were exposed to relatively high concentrations of such mutagens as sulphur-mustard gas, and that the cancer incidence among the workers was significantly high. We have been investigating the potential genetic effects of this gas at the protein level. In this report, presented are the results of erythrocyte enzyme activity measurement for variants demonstrating abnormalities in activity in 620 children born to the workers during employment and after termination. Using red cells preserved in liquid nitrogen, a 1 : 20 diluted hemolysate was prepared by the ICSH method. Activity of 9 different erythrocyte enzymes (TPI, PGK, AK1, LDH, GPI, PK, 6PGD, G6PD, HK) were determined by the method of Satoh *et al.* (1983)[1] and the mean value and the standard deviation (SD) were calculated. The number of tests varied by enzyme from 204 (HK) up to 620 (LDH and 6PGD), and the total number of tests performed was 4,336. Of the initial and newly collected blood samples, those whose activity values were 3 SD lower than the mean value were AK1 (1 out of 577 cases), LDH (4 out of 620) and PK (1 out of 236 cases). The low activity variant of AK1 showed normal profile in the heat denaturation experiment, while all 4 cases of LDH variant showed labile profiles. Study

of the enzyme activity of the parents of 5 cases has been completed and in all 5 cases identical variants of low activity were detected in one of the parents, and thus none of these 5 variants were due to mutations. Parents of the propositus with a low activity of PK could not be examined.

1) Satoh, C., Neel, J.V., Yamashita, A., Goriki, K., Fujita, M. and Hamilton, H.B. 1983. *Am. J. Hum. Genet.* **35**: 656

B33. Sensitivity to Ethylnitrosourea in Fibroblasts from Patients with Bloom's Syndrome: Takayuki KURIHARA, Minoru MIYAKOSHI and Masao INOUE (Cent. Lab., Kanazawa Med. Univ., Uchinada, Ishikawa)

Fibroblasts from patients with Bloom's syndrome (BS) have been investigated for the viability, sister-chromatid exchanges (SCEs) and chromosomal aberrations in *N*-ethyl-*N*-nitrosourea (ENU)-treated cultures. When the sensitivity was compared in terms of D_0 value as measured by colony forming ability, BS cells were sensitive to ENU twice as much as normal cells, but the different sensitivity to *N*-methyl-*N*-nitrosourea (MNU) between BS and normal cells was not observed. BS cells exhibited extremely high frequency of ENU-induced SCEs when compared with normal cells. However, BS cells were similar to normal cells in the induction of chromatid breaks by ENU treatment, and also in the reduction and recovery kinetics in the rate of semi-conservative DNA synthesis after ENU treatment. These results suggest that the probability that BS cells will remove O^6-ethylguanine is low as compared with normal cells.

B34. Ultraviolet Light Induced Mutation of Normal and Xeroderma Pigmentosum Lymphoblastoid Cells: Kouichi TATSUMI, Kazuko MIYAKE (Radiat. Biol. Cent., Kyoto Univ.), **Yukari SUGAI and Hiraku TAKEBE** (Dept. Exp. Radiol., Fac. Med., Kyoto Univ., Kyoto)

Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by severe photosensitivity and an increased incidence of skin cancer. Survival and mutation after ultraviolet (UV) irradiation, measured by the use of a microtiter plating technique, were compared between XPA3, diploid lymphoblastoid cells derived from a XP patient of complement group C, and HH4, diploid lymphoblastoid cells with normal excision repair capacity. Relative to normal cells, XPA3 cells were more sensitive to killing by virtue of a diminished shoulder and a steeper slope in the survival curve. XPA3 lymphoblastoid cells were also more sensitive than HH4 cells to mutation determined for 6-thioguanine resistance and for ouabain resistance, confirming the increased UV-mutability of XP somatic cells which had been originally reported for fibroblasts by Maher *et al.*

B35. Studies on the HLA (DR) Antigens in Neural Tube Defects: S. FUJISAWA, K. AOKI, K. SUZUMORI and Y. YAGAMI (Dept. Obst. Gynecol., Nagoya City Univ., Nagoya)

The T/t complex in the mouse is considered as a gene locus that would bring about neural tube defects (NTD). This locus is found to exist near the H-2 complex, the MHC of the mouse. Accordingly, due to similarities of the mouse's MHC to that of human being, it is thinkable that there exists a T/t complex near the HLA complex, especially the DR locus. Studies on the HLA-A, B, DR and MT loci of parents of NTD and their children with the intention of revealing the relationship between NTD and the HLA complex in human being were undertaken by us. Results obtained were as follows: 1) A significantly higher frequency of HLA-DR 5 was found in the mother of NTD as compared to the controls. 2) In a significantly higher frequency, one or two HLA-DR antigens were shared by the parents of NTD. 3) A significantly higher frequency of HLA-DR and MT homozygosity was found in the NTD as compared to the controls. These results indicated that the T/t complex in man exists near the HLA-DR and MT loci, hence, a significant relationship between the NTD and HLA complex was further ascertained.

B36. Analysis of Human Immune Suppression Gene Using Long Term Cultured T Cell Lines: Kenji HIRAYAMA, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

Low immune responsiveness of human peripheral blood lymphocyte to streptococcal cell wall antigen (SCW) is controlled by an HLA-linked single dominant gene through the induction of the antigen specific suppressor T cells. In this study, we established SCW specific helper T cell (Th) lines from both high and low responders by using Interleukin-2 prepared from the culture supernatant of the Gibbon lymphoma cell line MLA-144. These Th lines had characteristic cell surface markers of Th, Leu 1^+, 2^-, 3^+ and surface Ig^-. The proliferative response of Th lines to the several antigens presented by autologous or allogeneic monocyte (Mϕ) was examined. The Th lines from low responders as well as high responders showed proliferative response specific to SCW in the presence of allogeneic Mϕ which shared at least one HLA-DR antigen with T cell donors. Mouse anti-DR framework monoclonal antibody completely blocked the proliferation of Th lines. From these data, we concluded that the genetic restriction by HLA-DR antigens existed in the cooperation between low responder Th line and monocyte as well as high responder. Low responder monocyte had no defect in the antigen presentation to Th lines. HLA-MT antigens had no influence on the cooperation between them. These facts support that

no difference exists in the cooperation of Th cells with monocytes between high and low responder.

B37. Production and Characterization of the Human T Cell Hybridomas: Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

We have reported that low immune responsiveness to the streptococcal cell wall antigen (SCW) was controlled by an HLA-linked immune suppression gene (Is-SCW) through antigen specific suppressor T cells. In order to investigate the action of suppressor T cells specific to SCW, we tried to produce suppressor T cell hybridoma. 8 azaguanine resistant mutant of human T cell leukemia line CEM (CEM-AG^R) were hybridized with human T cells stimulated with SCW (CEM-AG^R : T cell = 1 : 2) using polyethylene glycol. Hybridomas were selected by selection medium containing azaserine and hypoxanthine for 3 to 4 weeks. About 200 T cell hybridoma clones were established and the probability of the production of T cell hybridoma was calculated as 2.30×10^{-5} for CEM-AG^R. Chromosome number was 93 ± 4 in hybridoma and 88 ± 1 in CEM-AG^R. Character of lymphokines produced by T cell hybridoma was checked by adding culture supernate of hybridoma to the assay system. We could establish 4 clones producing interleukin-2 (IL-2), which stimulates the proliferation of IL-2 dependent helper T cell line specific to SCW. Four clones were observed to enhance the proliferative response of peripheral blood lymphocytes (PBL) specific to SCW and other 3 clones had suppressive effect on the proliferative response of PBL to SCW. We can, thus, analyze the action of SCW specific helper or suppressor T cells by using these human T cell hybridomas.

B38. Suppressor T Cells Specific for both Cedar Pollen Antigen and IgE Isotype: Masahiko MUTO, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ.) **and Yozo SAITO** (Dept. Otolaryngol., Sch. Med., Tokyo Med. Dent. Univ., Tokyo)

We have reported that both the resistance to cedar pollinosis and IgE nonresponsiveness to cedar pollen antigen (CPAg) were controlled by an HLA-linked dominant gene. IgE nonresponsiveness to CPAg *in vitro* was mediated by Leu 2^+3^- suppressor T cells and we designated the HLA-linked dominant gene controlling IgE nonresponsiveness to CPAg as the immune suppression gene for CPAg (Is-CPAg). In this paper, we have investigated the specificities of suppressor T cells both for antigen and immunoglobulin isotype. Peripheral blood lymphocytes (PBL) from K.I. (responder to both CPAg and mite antigen

(MTAg)) were challenged with either CPAg or MTAg *in vitro* in the presence of T cells from M.M. (nonresponder to CPAg but responder to MTAg). T cells from M.M. completely abolished the IgE response of K.I. to CPAg but did not have effect on IgE response of K.I. to MTAg. The Leu 2^+3^- suppressor T cell is thus CPAg specific. In a cedar-allergic family, nonallergic father shows IgG response but not IgE response to CPAg. Child (C2) in that family is affected and shows both IgG and IgE response to CPAg. In order to examine the isotype (IgE) specificity of the suppressor T cell, PBL from C2 were challenged with CPAg *in vitro* in the presence of T cells from father. T cells from father completely abolished the IgE response without showing any suppressive effect on IgG response of C2 to CPAg. Thus it is evident that there are antigen specific and isotype specific suppressor T cells for CPAg.

B39. Immunogenetic Analysis of Leprosy III: Ikuo KIKUCHI, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Toshiharu OZAWA** (Natl. Inst. Leprosy Res., Tokyo), **Kiyotaka SANADA and Masanori KOSEKI** (Natl. Sanat. Tama-Zenshoen, Tokyo)

Genetic control of the clinical manifestation of leprosy was investigated using 66 unrelated patients (34 tuberculoid patients and 32 lepromatous patients) and 177 members of 47 multiple case families. In unrelated patients with leprosy HLA-DR2 and -MT1 were significantly increased (r.r.$=3.76$, $\chi^2=16.60$, r.r.$=4.09$, $\chi^2=11.10$, respectively) compared to healthy controls. Multiple case families with leprosy were analyzed by affected sibpairs method. 8 sibpairs shared two HLA haplotypes and 10 sibpairs shared one HLA haplotype. These haplotype distribution differed from the random distribution ($\chi^2=7.33$, D.F.$=2$, $p<0.03$). This method could not distinguish between the recessive model (g.f.$=0.45$) and the dominant model (g.f.$=0.16$) for the HLA-linked disease susceptibility gene for lepromatous leprosy (LL). We also analyzed the mode of inheritance for lepromatous leprosy by Morton's maximum likelihood scoring method. The hypothesis that lepromatous leprosy was controlled by a single recessive gene, was discarded because there was a significant difference between observation and expectation in 18 informative backcross families ($p<0.05$) and in 12 intercross families ($p<0.005$). The hypothesis of a single dominant gene for LL could not be discarded following the analysis of 15 informative backcross families ($p>0.05$) and 4 intercross families ($p>0.05$). These data supported simple dominant inheritance for LL. Furthermore it was demonstrated that antigen specific suppressor T cell (Leu 2^+) was present in a patient with lepromatous leprosy. All these observations suggested that an HLA-linked single dominant gene controlled the clinical manifestation of leprosy through T lymphocytes.

B40. 日本人集団における亜急性甲状腺炎・全身性紅斑性エリテマトーデス・mixed connective tissue disease および進行性全身性硬化症と HLA の相関：五島 寛・宇野久光・鬼沢 信・笹月健彦（東医歯大・難研・人類遺伝），**玉井 一**（九大・医・心療内科），**秋月正史・本間光夫**（慶大・医・内科）. **Associations between Subacute Thyroiditis, Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Progressive Systemic Sclerosis and HLA:** H. GOTO, H. UNO, S. ONISAWA, T. SASAZUKI (Tokyo Med. Dent. Univ., Tokyo), H. TAMAI (Dept. Psychosomat. Med., Kyushu Univ., Fukuoka) and M. AKIZUKI, M. HOMMA (Dept. Intern. Med., Keio Univ., Tokyo)

日本人集団中の亜急性甲状腺炎患者 89 名の HLA-A,B,C 抗原および全身性紅斑性エリテマトーデス (SLE) 患者 60 名，mixed connective tissue disease (MCTD) 患者 27 名，進行性全身性硬化症 (PSS) 患者 26 名の HLA-DR 抗原を NIH 標準リンパ球微量細胞傷害試験により検索した．亜急性甲状腺炎患者では，Bw35 抗原の著明な増加（抗原頻度 67.4%, r.r.12.6, $p<0.0001$）が観察された．Bw35 遺伝子と強い連鎖不平衡にある亜急性甲状腺炎の疾病感受性遺伝子を仮定し，その遺伝様式を Thomson と Bodmer の方法により推定すると，単純劣性モデルにほぼ一致した．亜急性甲状腺炎は単純劣性遺伝に従う可能性が示された．SLE では MTI が (r.r. 3.22, $p<0.005$), MCTD でも MTI が (r.r. 3.27, $p<0.05$), PSS は DR2 が (r.r. 3.33, $p<0.01$) 増加していた．また，これら 3 疾患の抗核抗体出現頻度をみると，SLE では ds- および ss-DNA, Sm, SS-A, SS-B, Ki 抗体が他の 2 疾患に比べて有意に増加していたが，これら自己抗体の陽性群と陰性群との間では，HLA-DR 抗原との相関に差が認められなかった．一方，抗 Scl-70 自己抗体は PSS 患者の 53.8% にのみ出現し，PSS 患者を Scl-70 抗体陽性群と陰性群とに分けた場合，陽性群は DR2 との高い相関 (r.r. 8.97) が認められたのに対して，陰性群では認められなかった．これは，抗 Scl-70 自己抗体の抗体産生免疫応答に DR2 が重大な関連をもっている可能性を示している．

B41. HLA Antigens and Environmental Factors in the Development of Behcet's Disease: Kenji TANAKA, Kenji KAJIYAMA, Shudo Nishigori, Shiro KAMEDA, Takashi IMAMURA (1st Dept. Med., Kyushu Univ.), **and Toru INOUE** (Dept. Ophthalmol., Kyushu Univ., Fukuoka)

To investigate gene-environment interaction in the development of Behcet's disease, analysis of HLA antigens and case control study were performed in 50 patients with Behcet's disease. Frequency of HLA-Bw51 (52.0%) was significantly increased in the patients as compared to that (16.0%) in the control subjects. In case control study, the incidence of tonsillitis was more frequent in the patients than in the controls, and so the use of public bath and a privy. The patients were frequently exposed to agricultural chemicals before onset of the disease. Daily ingestion of vegetable salad and milk was less frequent in the patients than in the controls. A multivariate analysis was performed for these selected variables. Correlation ratio obtained from discrimination analysis was 0.410. Highest partial correlation was observed between HLA-Bw51 and Behcet's disease. Eighty

per cent of patients with Behcet's disease could be discriminated from control group by this analysis.

B42. Genetic Control of the Humoral Immune Response to the HBs Vaccine in Man: **Yasuharu NISHIMURA, Michiko DOBASHI, Takehiko SASAZUKI** (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Hiroshi MOCHIZUKI and Motoi MORIMOTO** (Dept. Dent. Pub. Health, Nihon Univ. Sch. Dent., Matsudo)

Association between HLA or Gm and immune responsiveness to the vaccine for the surface antigen of the hepatitis B virus (HBs vaccine) was investigated in 97 healthy unrelated adult volunteers (81 males and 16 females, 25–30 years). They were immunized subcutaneously with 40 μg of HBs vaccine on month 0, 1, 3 and 6. HBs vaccine was purified from HBs positive pooled human plasma and was conjugated with aluminium hydroxides after inactivation by treating with formalin and heating. Blood samples were separated from volunteers on month 1, 2, 3, 4.5, 6, 7, 12 to check the antibody to HBs antigen by passive hemagglutination test (PHA). Forty four (45.4%) volunteers responded to HBs vaccine at month 2. The number of responders increased to 59 (60.8%) and 80 (82.5%) at month 3 and 7 respectively. The phenotype frequency of HLA-A2 is significantly increased in nonresponder group as compared with responder group (52.8% *vs.* 22.7%, $p<0.005$) at month 2. Significant increase of HLA-A2 was also observed at month 3 (55.6% *vs.* 29.5%, $p<0.02$), but the association disappeared at month 7. Phenotype frequency of the Gm haplotype afb^{01345} was, on the other hand, increased in responder group as compared with nonresponders (27.3% *vs.* 9.4%, $p<0.03$) with statistical significance only at month 3. These observations suggested the existence of the genetic control of the immune responsiveness to HBs vaccine by both HLA-linked and Gm-linked genes.

B43. HLA in Takayasu Disease: **Fujio NUMANO, Ichiro ISOHISA, Junko MITANI, Tomoe KUROIWA** (Dept. Int. Med., Tokyo Med. Dent. Univ.), **Michiko EGAMI and Takehiko SASAZUKI** (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo)

Recent studies on the pathogenesis of Takayasu disease have focussed on the participation of genetic factors and our HLA analysis has confirmed the close relationship between Takayasu disease and HLA Bw52-Dw12 (Tissue antigen 12 : 246, 1978; 14 : 177, 1979). These findings raised the question of whether or not these genetic factors are associated with immune response (Ir) genes, as Takayasu disease is postulated to be auto-immune disease, and the question of what clinical characteristics are reflected by this haplotype.

In an attempt to investigate the association of Ir gene with the disease, HLA-DR, MT and MB antigens were studied in 52 patients with Takayasu disease and the data compared with findings in healthy Japanese. However, there were no statistical differences between the two groups in the frequencies of all these antigens. Clinical conditions and laboratory data of Takayasu disease observed for 85 ± 3 months were compared between 29 patients with (positive group) and 39 patients without HLA Bw52-Dw12, among 82 patients with this disease. Blood sedimentation rate and CRP revealed statistically significant high figures in the positive group as compared with those in negative group. Also statistically high frequencies in the complications of hypertension, aortic insufficiency and visual disturbance were recorded in the positive group. From these data, we postulated that genetic factors in Takayasu disease may be located more closely to B loci than to DR loci which accelerate an inflammatory state to progress these morbid conditions.

B44. Blood Types in Takayasu Disease: Fujio NUMANO, Junko MITANI, Tokuko SAKAMOTO (Dept. Int. Med., Tokyo Med. Dent. Univ., Tokyo), **Tokiko MIYASAKI and Hideo MATSUMOTO** (Dept. Legal Med., Osaka Med. Coll., Osaka)

Much attention has been recently drawn to the possible association of genetic traits with Takayasu disease. To search association between a variety of genetic traits and this morbids condition, blood types of ABO, MNSs, P, Rh-Hr, Kidd, Duffy, Diego, Kell, Lutheran, Phenotypes of Hp, Tf, Gc, Gm, Km and Pi, and red cell enzymes of AP, PGM, 6-PGM, EsD, S-GPT, S-GOT, UMPK, GLO and ADA in 83 patients of Takayasu disease were investigated, comparing with those in normal Japanese. Statistically high gene frequencies were confirmed in MS($p=0.006$) of MNSs, R^3(0.019) of Rh-Hr, B(0.037) and D(0.003) of Tf, and M_3(0.014) of Pi in patients group. Statistically low gene frequencies were also found in 1S(0.014) of Gc and afb^{01345}(0.002) of Gm in patients. These figures were more striking in group of patients without haplotype of HLA Bw52-Dw12. These data suggest that genetic factors may participate in the etiology of Takayasu disease, which may be located in chromosome No. 1, 4 and 14 besides No. 6.

B45. Biochemical Analysis of the Polymorphism of HLA-D Region Products Associated with DYT, DKT2 and Dw4: Hiroshi KOJIMA, Naoshi ISHIKAWA, Shuichi HOKIN, Hitoshi IKEDA, Yumi OHKOSHI, Akemi WAKISAKA, Yuko KIKUCHI and Miki AIZAWA (Dept. Pathol., Hokkaido Univ. Sch. Med., Sapporo)

HLA-D region products associated with DYT, DKT2 and Dw4 cause mutual MLC, although their DR and MB antigens are equally typed as DR4 and MB3. In this report, we investigated HLA-D region products of these three HLA-D specificities by using 2-dimensional gel electrophoresis. *HLA-D/DR* region-homozygous B lymphoblastoid cell lines EBV-Wa (DR4, MB3, DYT), KT2 (DR4, MB3, DKT2), and ER (DR4, MB3, Dw4) were analysed for their class II molecules. The results showed that their DRα and MBα chains were identical, but all of their DRβ and MBβ chains were structurally different. These findings reveal that DR4 and MB3 molecules associated with DYT, DKT2 and Dw4 are not identical. This indicates that the *HLA-D* region has more allelic genes than currently recognized.

B46. Analysis of Human Major Hitocompatibility Complex (MHC) Class II Antigen Systems: Manabu SUZUKI, Toshio YABE, Ryozaburo MUKAI (Dept. Hum. Genet., Univ. Tsukuba), **Masahiro SATAKE, Takeo JUJI** (Dept. Blood Transfus., Tokyo Women's Med. Coll., Tokyo) **and Hideo HAMAGUCHI** (Dept. Hum. Genet., Univ. Tukuba, Ibaraki)

The human MHC class II genes are mapped on the HLA-D/DR region of chromosome 6. At least three kinds of class II antigens, *i.e.*, DR, DC, and SB antigens, are expressed on one D/DR region homozygous B cell line. We have previously reported that both MB (TB, DC) and MT3 antigens are different from DR antigen in D/DR region homozygous B cell lines. This experiment was performed to clarify how many kinds of human class II molecule are expressed on one D/DR homozygous B cell line. We identified MB1, MB2, TB21 molecules by two-dimensional gel electrophoresis. Both heavy and light chains of these molecules are different from DR antigen and show genetic heterogeneity. In contrast, MT3 antigen has the same heavy chain as DR but the light chain different from DR. In a DR7 homozygous cell line, at least three kinds of human class II molecules, DR7, MB2, and MT3, are expressed. In a DRw6 homozygous B cell line, MT2 antigen is identified. It has the same heavy chain as DR but the light chain different from DR. Because biochemical property of MT2 is very similar to that of MT3, MT2 and MT3 antigens appear to be the allelic products of the same locus. MT3 antigen system is distinct from SB antigen system because no recombination between MT2 or MT3 and DR antigens has been reported yet, while SB locus is separated 2 centimorgans from DR locus.

These data indicated that there are at least four kinds of human class II antigen system. They are DR, MB, SB, and MT3 antigen systems.

B47. Genetics of SB Locus in the Japanese Population: Yasuharu NISHIMURA, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Norikazu YASUDA** (Div. Genet., Natl. Inst. Radiol. Sci., Chiba) **and S. SHAW** (Immunol. Branch, NIH, Bethesda)

SB antigen is a new HLA class II antigen and the SB locus has been mapped between HLA-D and GLO-locus by Shaw *et al.* in 1980. We have investigated the genetics of SB locus in 120 unrelated healthy Japanese. Five SB antigens were typed by primed lymphocyte test using two independent SB primed lymphocytes for each SB antigen presented by Dr. Shaw. In brief, peripheral blood lymphocytes (2×10^5) treated with mitomycin C were cultured with SB primed lymphocytes (2×10^4) for 3 days at 37°C. The proliferation of primed lymphocytes was measured by counting incorporated [^{3}H]thymidine into primed lymphocytes. The distribution of the response of primed lymphocyte to panel cells was analyzed by cluster analysis and the cut-off point for typing response was decided. The response of two independent primed lymphocyte for same SB antigen was concordant. The distribution of the SB phenotypes in 120 panels fitted Hardy-Weinberg equilibrium ($0.05<p<0.10$). Gene frequency of SB alleles was estimated in the Japanese population as follows: SB1 0.00, SB2 0.195 ± 0.027, SB3 0.070 ± 0.017, SB4 0.091 ± 0.019, SB5 0.335 ± 0.034 and SB blank 0.308 ± 0.035. Absence of SB1 allele, decrease of SB4 allele and increase of SB5 allele are characteristic feature of SB system in the Japanese population as compared with Caucasians. Each SB alleles is in linkage disequiribrium with some HLA-DR alleles.

B48. Polymorphism of the HLA-DR4 Molecule: Kikuo TSUKAMOTO, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The molecular composition and functions of HLA-Dw4, DYT and DKT2 were analyzed. Dw4, DYT and DKT2 were mutually stimulatory in mixed lymphocyte culture reaction (MLR), but all typed as DR4 in B cell serology. Metabolically L-[^{35}S]methionine labeled membrane protein solubilized from HLA-D homozygous B lymphoblastoid cell lines, ER (DR4, Dw4), HA (DR4, Dw4), Wa (DR4, DYT), KT9 (DR4, DYT), KT2 (DR4, DKT2) and KT13 (DR4, DKT2), were used as antigen source. Class II antigens were prepared by immunoprecipitation using anti-DR monomorphic monoclonal antibody, HU-4, and

analyzed by two dimensional polyacrylamide gel electrophoresis (2D-PAGE). Class II molecules from these cell lines were composed of one set of polypeptide groups, α chains (M.W. 32–34K) and β chains (M.W. 28–30K). Comparison of 2D-PAGE profiles showed that the α chains from these lines were identical in their mobility, but the β chains from these lines were different from each other in charge. The β chains from DYT cell lines were more basic than that from Dw4, and the β chains from DKT2 were most acidic. This was confirmed by the further analysis of non-sialidated or non-glycosilated precursor polypeptides from the thirty minutes pulse labeled or tunicamycin treated cell lines. HU-4 can block the mutual MLR between Dw4, DYT and DKT2, and can abolish completely the immune proliferative response of peripheral blood lymphocyte to the CSW antigen *in vitro*. Moreover, in this immune response, T cell (lines) can recognize the difference of these three molecules. These results indicate that the class II molecules from DR4 homozygous cell lines bear the DR4 antigenic determinant and that the different epitopes of the two β chains are responsible for the MLR between Dw4, DYT and DKT2, and for the modulator of the cooperation between T cells and monocytes to respond to SCW antigen.

B49. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. VI. The Linkage Relationship between a New Polymorphic Membrane Polypeptide and HLA Loci: **Kimiko YAMAKAWA, Yasuko YAMANOUCHI, Ikuko KONDO, Hideo HAMAGUCHI** (Dept. Hum. Genet., Univ. Tsukuba), **Michiko YOSHIOKA** (Dept. Legal Med., Osaka Med. Sch., Takatsuki), **Atsuo NOGUCHI** (Dept. Immunol., Univ. Tsukuba), **Masanao SHIBASAKI** (Dept. Pediatr., Univ. Tsukuba, Ibaraki) **and Norikazu YASUDA** (Div. Genet. Natl. Inst. Radiol. Sci., Chiba)

A cellular polypeptide with mol. wt. of 44,000 has three common phenotypes detected in peripheral blood lymphocytes by non-equilibrium pH gradient electrophoresis. Family and population studies indicate that the three phenotypes are determined by two common alleles at a single autosomal locus. In a Japanese population, the gene frequencies of the two alleles were 0.67 and 0.33, respectively. The 44K polypeptide was enriched in the microsomal fraction but not detected in the cytosol fraction, suggesting that the polymorphic polypeptide is a membrane protein. Therefore, it is temporarily designated membrane 44k polypeptide (M44k polypeptide). We examine the linkage relationship between M44k polypeptide and HLA loci by the sequential linkage analysis of Morton (1955), and Steinberg and Morton (1956). There are 19 informative families with a total of 50 children. The maximum lod score for linkage of the M44k polypeptide and HLA-B loci was 3.12 at $\theta=0.08$. M44k polypeptide was also observed to exist in granulocytes and fibroblasts. Furthermore, no correlation was observed between M44k polypeptide and HLA

class I antigens among unrelated individuals. These data indicate that M44k polypeptide locus is a new genetic marker linked to HLA loci.

B50. A Possible 'Silent' Allele at the Locus for Properdin Factor B: Katsushi TOKUNAGA, Keiichi OMOTO (Dept. Anthropol., Univ. Tokyo), **Yasushi YUKIYAMA** (Dept. Med. Phys. Ther., Univ. Tokyo, Tokyo), **Hiroo SAJI, Estuko MARUYA** (Kyoto Red Cross Blood Cent., Kyoto) **and Michiyo SAKURAI** (Ohtsu Red Cross Hosp., Ohtsu)

At least three BF alleles, *BF*S*, *BF*F*, and *BF*FT* (*BF*F075*) have been identified in Japanese. Recently, we found an informative family indicating the existence of a BF silent or quantitatively deficient allele (*BF*Q0*). Three generations with eight members were studied for BF, C2, C4, HLA (A, B, C, and DR), and GLO polymorphisms. Four members were considered to be heterozygous for B deficiency. The *BF*Q0* segregated with the haplotype *A*w24-C*w1-B*w54-C4A*J3B*Q0-C2*C-DR*w8-GLO*2*. Strong linkage disequilibrium among *C*w1*, *B*w54* and *C4A*J3B*Q0* has been reported. Antigen concentrations and functional levels of B and a few other components were determined. All of the probable *BF*Q0* heterozygotes showed low levels of B. As far as we know, this is the third report which supports the existence of a BF silent allele. The haplotype carrying *BF*Q0* in the present study is different from those reported previously. No homozygous deficient individuals have been found thus far. The frequency of BF silent allele(s) may be considerably low and homozygous deficient individuals may not survive.

B51. Studies on Complement Deficiencies in Blood Donors in Osaka Area of Japan, Part 2: Keiji YOSHIMURA, Yasuo FUKUMORI, Shiro OHNOKI, Yasuto OKUBO, Hideo YAMAGUCHI, Masayoshi TANAKA (Osaka Red Cross Blood Cent., Osaka), **Yohji AKAGAKI and Shinya INAI** (Dept. Clin. Pathol., Osaka Med. Coll., Takatsuki)

In the previous paper we reported that five of the 52,175 blood donors were suspected as C7 deficiency and two were C8 deficiency. The hemolytic assay of the complement components and immunochemial analysis of complement proteins in these sera showed that these donors were identified as C7 deficiency or C8 deficiency. These donors were all in good health. The family studies on these C7- or C8-deficient donors revealed that the mode of inheritance of these traits is autosomal co-dominant. 45 of another 48,627 blood donors were identified as C9 deficiency. Moreover, two of them were suspected as C5 deficiency and the other two were as C6 deficiency by immunochemical analysis.

B52. Estimation of Heritability for Hypothenar and Thenar Patterns on the Palm and Their Genetic Association: Kazumichi KATAYAMA (Dept. Phys. Anthropol., Fac. Sci., Kyoto Univ., Kyto)

In order to evaluate the relative importance of hereditary factors in palmar dermatoglyphic patternings, palm prints of a total of about 600 Japanese families, which were a part of Furuhata's collection in the possession of the Department of Forensic Medicine, Tokyo Medical and Dental University, were analyzed on the basis of the multifactorial inheritance model proposed by Falconer (1965). Based on Falconer's model, the heritability values were estimated at $63.9 \pm 9.0\%$, $75.8 \pm 19.8\%$, and $79.7 \pm 10.5\%$ for hypothenar radial loops, hypothenar ulnar loops, and thenar true and vestigial patterns, respectively, indicating a considerably strong genetic factors in palmar dermatoglyphic patternings. It was found that there was an appreciable negative phenotypic correlation between hypothenar and thenar patterns (significant at the 0.05 level only in females), *i.e.*, thenar true and vestigial patterns appeared at lower frequency among affected individuals for hypothenar true patterns than among unaffected ones. An appreciable negative genetic correlation was also recognized between both the patterns and the correlation coefficient was estimated at -0.1339, suggesting that the phenotypic correlation was mainly caused by the genetic correlation.

B53. 皮膚紋理の再生・再編成に関する研究：松井一郎（愛知コロニー・発達障害研），**松井　猛・町田拓也**（信州大・医・整外），**佐々木喜一郎**（日本農村医学研）．**Regeneration of Ridged Skin in Macaca Fuscata:** I. MATSUI (Inst. Develop. Res., Aichi Pref. Colony, Kasugai), T. MATSUI, T. MACHIDA (Dept. Orthoped., Shinshū Univ., Matsumoto) and K. SASAKI (Inst. Rural Med. JPN, Saku)

指尖，手掌の損傷を扱う手の外科領域で，保存的な創開放療法で隆線皮膚の再生現象が報告されている．皮膚紋理の再生と再編成を明らかにするために macaca fuscata の手掌・指尖の切除実験を行った．切除形態は大と小，円形または紡錘形，隆線走行に平行と直角，pad 切除の有無を組み合わせた．切除創は開放とし，8 週後に紋様と隆線形態を観察した．

結果は，1) 隆線の完全な再生はみられなかったが，紋型の修復は切除時の隆線走行の条件と密接に関係し，隆線走行に直角の切除では治癒後隆線が中断（瘢痕形成）する．斜または平行の切除では紋型保持の方向で修復がおこる．2) 隆線と平行の切除では欠損部を埋めるかたちで紋理の再編成がおこる．小さな欠損ではもとの紋型が維持されるが，pad 切除を含む大きな欠損では治癒後にきわめて複雑な紋様となる．pad 切除を伴わない場合（三叉部分の切除）では，もとの紋様が維持される．3) 創治癒後の隆線形態には大きな変化が認められ，隆線幅の増大，変形，癒合などが一般的であった．

B54. Inheritance of the Absence of Palmar Digital Triradius d.: Michio OKAJIMA (Dept. Forens. Med., Tokyo Med. Dent. Univ., Tokyo) **and Chizuko IWAYA-NAGAI** (Dept. Physic. Anthropol., Univ. Tokyo, Tokyo)

The absence of palmar digital triradius d was examined in 2,683 Japanese families, including both parents and 3,305 sons and 2,852 daughters. The frequency of the trait on the left palm was 0.56% and significantly higher than on the right, 0.16%. No sex difference was found. The trait appeared in 73 (0.6%) out of 11,523 persons in one or both hands. Among 2,644 families in which both parents were not affected, the absence of d was observed in one child in 28 families and two in two families. On the other hand, one child affected with absent triradius d was found in two among 39 families, in which one parent was affected. Thus, the genetic effect on this trait was suggested by the higher incidence in relatives of affected individuals. However, the recurrence ratio was not high, *i.e.*, 2.2% (2/92) in children, 3.1% (2/64) in parents and 3.7% (2/54) in sibs of the affected, and these are too low to estimate a simple Mendelian inheritance with high penetrance. The recurrence in relatives of bilaterally affected was 8.7% (2/23) and higher than in those of unilaterally affected, 3.1% (6/195). From these findings, a multifactorial mode of inheritance was suggested for the occurrence of this trait.

B55. 脳障害を伴った乳児期双生児の発達差異について：岡田良甫・長谷川要子・四家正一郎（金沢医大・小児）.

乳児期脳障害のみられた一卵性 13 組，二卵性 8 組に経時的発達検査を行い，知的発達と粗大運動発達，社会性の発達を follow して次の結論を得た．1) 治療および訓練によって正常に回復した症例については，一卵性，二卵性いずれもゴールに達するまでの間に 1～2 歳ころの経過中に一過性の知的発達，運動発達に差異が認められたが，ゴールに近づくにつれて格差は消失し，目的達成時点では，両者同等の発達を示した．2) 障害治療中の環境的因子（家族の扱い方，治療内容など）によって一過性の発達差異を生ずることがある．3) 知的活動性の発達差を生ずる原因の一つとして，34 週以前の在胎週数という生物的因子も関与していると考えられた．

B56. アルコール依存症およびその他の精神障害の双生児研究：山田一朗・浅香昭雄・逸見武光（東大・医・精神衛生）. **Twin Study on Alcohol Dependence and Other Psychiatric Disorders:** Kazuaki YAMADA, Akio ASAKA and Takemitsu HEMMI (Dept. Mental Health., Sch. Med., Univ. Tokyo, Tokyo)

病院要覧（79 年版）および医育機関名簿（82～83 年版）を用いて，全国の大学附属病院，単科精神病院，100 以上の精神科病床を有する一般総合病院総計 1,316 個所を選出し，83 年 3 月～4 月にかけてアンケートを郵送した．質問内容は，まず双生児症例の有無を問い，次にその症例がアルコール依存症（以下ア症）と診断されているかどうかを聞く形とした．83 年 11 月 1 日現在，812 個所

の医療機関から回答があった（回収率 63.7%，ただし転居先不明 41 を除く）．そのうちア症の発端双生児は 14 組，それ以外の精神障害の発端双生児は計 99 組含まれていた．症例のあった病院に対しては性別，年齢，卵性（推定），診断名，双生児間での症状の一致の程度，現在入院状況，情報収集の可能性，職業ないし職歴，結婚歴，家族歴（とくに家族内遺伝負因）等の情報を電話により得た．当該疾患による入院歴の有無を判断の基準とし，一致率を計算した．ア症では一卵性 70%（7/10），二卵性 50%（1/2），精神分裂病では，一卵性 60%（30/50），二卵性 50%（5/10），また躁鬱病では，一卵性 40%（4/10）という値が得られた（いずれも proband-wise）．現段階では，卵性診断や，それぞれの疾患の診断基準も明確ではないので，今後より詳細なデータ収集が必要である．とくに，一卵性の不一致例に着目し，疾患の発現に関与する環境要因を中心に分析していきたい．

B57. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. VII. Application to the Diagnosis of Zygosity: Ikuko KONDO, Kimiko YAMAKAWA, and Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Using two-dimensional(two-D) gel electrophoresis developed by O'Farrell (1975) with some modification, we have detected 8 polymorphic polypeptides, *i.e.*, C100k, LC64k, C49k, C40k, C38k, C33k, C31k and Mt68k, in lymphocyte proteins. Family and population studies indicate that each polymorphic polypeptide is determined by two common alleles at a single autosomal locus and that the gene frequencies of the commonest alleles of these polypeptides range from 0.940 to 0.532 in a Japanese population. We applied these polymorphic polypeptides to the diagnosis of zygosity in 18 twin pairs (17 pairs of same sex and one pair of opposite sex). Phenotypes of lymphocyte proteins derived from 2 ml of peripheral blood of 18 twin pairs and their parents were examined by two-D gel electrophoresis. Two phenotypes out of 8 polymorphic polypeptides were discordant in one pair of opposite sex. On the other hand, the phenotypes of 8 polymorphic polypeptides were perfectly concordant in 17 like-sexed twin pairs. The probability of monozygosities calculated by the formula of Essen-Möller (1941) [Pr(MZ)], using the phenotypes of these polypeptides in 17 twin pairs and their parents, ranged from 0.998 to 0.875 and were over 0.95 in 76% of twin pairs studied. The Pr(MZ) based on the phenotypes of blood groups (ABO, MN, Se) and isozyme groups (phosphoglucomutase-1, acid phosphatase, esterase D, adenosine deaminase) ranged from 0.998 to 0.886 in 17 pairs of same sex and were over 0.95 in 80% of twin pairs of same sex. These data indicate that genetic analysis of cellular proteins by two-D gel electrophoresis is useful for the diagnosis of zygosity to a similar extent of that by means of blood and isozyme group systems.

B58. 一卵性双生児組内の免疫グロブリン値の差をもたらす要因についての検討：佐藤幸男・岡本直正（広島大・原医研・遺伝），**今村展隆・久住静代・藏本　淳**（同・内科），**渡辺正治・務中昌己・栗原　登**（同・社会医学・生物統計）．**Study on the Environmental Factors Which May Have Influence on the Immunoglobulin Value in Monozygotic Twins:** Y. SATOW, N. OKAMOTO, N. IMAMURA, S. KUZUMI, A. KURAMOTO, S. WATANABE, M. MUNAKA and M. KURIHARA (Res. Inst. Nucl. Med. Biol., Hiroshima Univ., Hiroshima)

双生児組内での種々な量的形質の変化を指標として遺伝と環境要因のかかわりあいの研究を行っているが，今回は免疫グロブリン(Ig)と種々な環境要因との関係について検討を行った．例として原爆被爆の条件とその影響の有無についての検討をあげると，14 組の一卵性双生児組内での被爆状況は症例によって異なり一様ではない．したがって種々な被爆条件下の，全体の双生児組内の Ig 値の差を変数とみなして 95% 信頼限界を求め，その限界域から離れている症例について検討した．同じ被爆条件下にある双生児組内では Ig 値の差が少なく異なる条件下にある組では Ig 値に差のある傾向がみられたが例外もあり，一定の方向づけは現在では困難と思われた．被爆条件の異なる双生児組内の少数例のモノクローナル抗体によるリンパ球サブセットの解析も同様の結果を示した．一卵性双生児の HLA A, B, C 座の頻度は，日本人の平均的ハロタイプと差を示さなかった．（本研究は文部省科研一般 C 58570965 の援助によった．）

B59. Effects of Parental Age and Birth Order in Motor Neuron Disease: Keiko FUJIKI and Kiyotaro KONDO (Dept. Neurol., Tokyo Metrop. Inst. Neurosci., Tokyo)

Although the cause of motor neuron disease (MND) is still obscure, evidences suggested that a complex interplay of genetic and environmental factors underlies its pathogenesis. The present study attempts to find a clue whether intrauterine factors predispose to subsequent development of MND by evaluating effects of the birth order and the age of parents at the birth of the victims on the risks to the disease.

Material and methods. Family information in 309 cases of MND and 244 their spouses as the controls were collected from 3,255 death certificates with MND and muscular atrophies retrieved out of 700,438 deaths in Japan in 1965, for which 51 items of clinical information were collected. Seven variables were calculated based on the family information for each case and control.

Results. Parental age: In all age groups of parents, number of the cases did not differ significantly from the expected number in either sex. Average parental ages were same. Birth order: In all birth order groups, number of the cases did not differ from the expected number in either sex. Average birth order were identical. The first and the pooled subsequent birth orders were compared disclosing that the primogeniture cases were deficient in the males. Sib size: No difference was observed in each sib size group in either sex. Birth interval: There was no difference in each birth interval group. The average interval in the males was significantly greater than that of the controls, however.

B60. Chromosome Studies in Couples with Recurrent Spontaneous Abortions: Kanichi SOH, Nobuyoshi OZAWA, Shinji SATO, Toshifumi TAKABAYASHI, Akira YAJIMA and Masakuni SUZUKI (Dept. Obst. Gynec., Tohoku Univ., Sendai)

An relative high incidence of abnormal parental karyotypes, especially balanced chromosome translocation, has been recently reported in literatures. However, the frequency of it varies widely in reported series. The reported incidence of balanced chromosome translocation in couples with histories of recurrent abortions ranges from 0% to 50%. For this reason the cytogenetic investigation of a sample of 35 married couples who had experienced two or more spontaneous abortions was carried out. There was no apparent gynecological cause for recurrent spontaneous abortions in any couples of our sample. Chromosome karyotypes were prepared from peripheral lymphocytes and stained by the technics for G-banding. Balanced translocations were found in 14.3% (five couples) of these 35 couples (7.1%, individuals). Four cases are Robertsonian translocations, t(13q14q), t(13q22q), t(14q21q) and t(21q21q), one case is reciprocal translocation, t(14;15)(q24;q24). With regard to past histories, for 14 couples with two spontaneous abortions, two (14.3%) had balanced translocations; for 16 couples with three spontaneous abortions, two (12.5%) had balanced translocations; and for five couples with four or more spontaneous abortions, one (20%) had a balanced translocation.

B61. Prenatal Diagnosis of Genetic Disorders: An Analysis of 729 Cases during the Last 13 Years: Kaoru SUZUMORI, Katsuhide IMAIZUMI, Setsuo OKADA, Yoshiaki YAGAMI (Dept. Obst. Gynec., Nagoya City Univ., Nagoya)

During the 13-year period between Oct, 1971 and Sep, 1983, 729 patients underwent midtrimester amniocentesis for genetic disorders at the Nagoya City University Hospital. Through these experiences, we assessed the frequency of abnormalities, safety of the procedure, and diagnostic accuracy. Since 1973, each patient had a ultrasound examination done on the day of the amniocentesis for placental localization, fetal position, confirmation of gestation, and screening for anomalies. Recently, real-time guidance was added, and 37% of the amniocenteses were done with real-time guidance. The risk of spontaneous abortion was reduced by the use of ultrasound, especially by the real-time guidance. Serious chromosomal or metabolic abnormalities were diagnosed in 44 fetuses. In addition, 5 fetuses were determined to be male in the sex-linked group. These parents elected termination of the pregnancies. There were 2 diagnostic errors (one: metabolic disease, one: chromosome). The karyotyping error rate was 0.1%. Follow-up data were obtained by direct examination or telephone, about the date of birth of the child, whether there were any birth defects, whether the child had had any neonatal problems, and whether the patient had had any obstetric complications. A response rate was 92.2%. We con-

clude that midtrimester amniocentesis is a highly reliable and accurate procedure that does not significantly increase the risk of fetal loss and injuries.

B62. Follow-up Study of Genetic Counseling (XI). Our Experience in Fukui: Norio FUJIKI, Masuji MORITA and Kazuo MANO (Dept. Intern. Med., Fukui Med. Sch., Fukui)

We have performed genetic couseling at Fukui Health Authoritative and our university department for two years since October 1981. Seventy-four cases were analyzed, compared with cases previously counseled in Kyoto (898 cases) and Nagoya (1,105 cases) areas.

Significantly more persons came to the couseling through the local health authoritatives in Fukui than the other areas ($p<0.01$), while mass media has participated less ($p<0.01$). The most common motive for counseling was problems on the recurrence risk of illness. Many counselees came at the time of marriage (48.5%) and of pregnancy (24.4%). The contents of counseling were 1) hereditary diseases (31.9%), 2) constitutional diseases like mental retardation and psychosis (20.2%), 3) consanguinity (19.1%), 4) malformation (12.8%). Compared with the other areas, hereditary disease was significantly frequent problems ($p<0.01$) and non-genetic disease (so-called nonsense code) infrequent ($p<0.01$) in Fukui, which suggests that genetic problems have latently existed without reasonable solution or that good understanding of genetics has spread in public gradually. The questionnaire survey among general inhabitants (274 persons) and medical (163) and nursing (187) students in Fukui revealed that they had sufficient interests in genetics but that their information was not enough to banish misunderstanding and prejudice against heredity.

B63. Frequency of ALDH-I Deficiency among Mentally Ills: Akio ASAKA (Dept. Mental Health, Univ. Tokyo, Tokyo), **Shoji HARADA** (Inst. Community Med., Ibaraki) **and Bunnoshin ISHIKAWA** (Utsunomiya Mental Hosp., Tochigi)

Frequency of ALDH-I deficiency among patients at a mental hospital was examined. Subjects were 103 alcoholics, 86 schizophrenics, 47 drug dependents and 45 patients with affective psychosis. Frequencies were 1.9%, 41.9%, 48.9% and 24.4% in the above order, respectively. As the frequency in 105 normal controls was 41.0%, that of alcoholics was extremely low, indicating that it is necessary for alcoholics to have non-deficient ALDH-I and to be able to drink too much. On the contrary, those who show deficient ALDH-I cannot drink heavily because of high concentration of acetoaldehyde in the blood, which causes side effects or flushing symptoms. The frequency is a little higher in drug dependents than in controls, suggesting that those subjects may consist of two groups, that is, one

is dependent on drugs only and the other on drugs as well as alcohol. It is interesting to note that the frequency was comparatively lower in patients with affective psychosis, indicating that patients with unipolar depression were included in this sample, whose genetic backgrounds are said to be partly overlapped with those in alcoholics. It is desirable to devide these patients into two subgroups, unipolar and bipolar ones, and to compare with each other. Alcoholism and drug dependence are bio-psychosocial disorders. We attempted to analyse family backgrounds among the other sample consisting of 100 alcoholics and 50 toluene dependents. No difference was observed between the two groups as to family backgrounds. Therefore, it was again suggested that whether they became alcoholic or drug dependent was mainly due to the difference of ALDH-I isozyme pattern.

B64. Analysis of the Specific Proteins in Patients with Systemic Lupus Erythematosus (SLE) III: Fumitaka MORITO, Akihide OHTA, Toshiro NAGAYOSHI, Hidetoshi KANEOKA and Masaya YAMAGUCHI (Dept. Intern. Med., Saga Med. Sch., Saga)

The development of SLE is thought to be concerned with the genetic factors, probably associated with the system of multifactorial inheritance. The variant genes which have submajor effects on the development of SLE may produce some particular substances. We analyzed the protein components of lymphocytes and sera from patients by two-dimensional gel electrophoresis, and performed family studies about SLE specific proteins, HLA class I antigen and immunoglobulin allotype (Gm). Among SLE specific three variant polypeptides, expression of two variant spots with mol. wt. 70,000/PI 7.0 (Lp. 1) and mol. wt. 41,000/PI 6.0 (Lp. 17) in the PHA-stimulated lymphocyte proteins were inherited with autosomal recessive mode, and that of the variant polypeptide with mol. wt. 31,000/PI 5.8 (S. 1) in sera with autosomal dominant mode. These SLE specific spots were present in 70–89% of SLE patients, and in 20–38% of normal controls. The frequencies of possessing these proteins in the relatives were in the intermediate range (47–72%). Much of SLE patients (57%) have all of these three proteins, but rarely normal controls. The relatives had more these spots than normal controls. In Gm analysis, SLE patients and the relatives with Gm $ab^{085}st$ genes showed some immunological abnormality and interestingly the high frequency of anti-DNA antibody. Also the relatives with two or more of SLE specific proteins seemed to produce anti-DNA antibody. These data strongly suggest that these variant polypeptide spots might be concerned with the genetic factors contributed to the development of SLE. However, it is possible that the development of SLE might be affected by additional genetic factors and/or environmental factors.

B65. アカタラセミアマウスの赤血球カタラーゼの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）. **Analysis on Blood Catalase of Acatalasemic Mice by Isoelectric Focusing:** Yukinori SATO, Yukiko TANAKA, Masana OGATA (Dept. Public Health, Okayama Univ., Okayama)

アカタラセミアウスの残余カタラーゼは活性が熱に対して不安定であることから，正常のカタラーゼ分子とは構造上の変異が推定されている．この確認のためにアカタラセミア肝カタラーゼの等電点電気泳動による分析につづいて，残余カタラーゼが正常の約 2.4% と少ないアカタラセミアマウスの赤血球カタラーゼについて調べた．正常，アカタラセミア同型接合体，ヒポカタラセミア同型接合体，アカタラセミア異型接合体各 3 匹より採血し，7°C, pH 3～10 のアガロース等電点電気泳動法で分離し，kI 染色を施した．その結果，各バンドの中心点の pH の平均値で比較すると，アカタラセミアマウス赤血球カタラーゼの等電点は正常と比べてアルカリ側に存在し，正常マウス赤血球カタラーゼはやや酸性側に近くバンドが形成される傾向があった．また，ヒポカタラセミア同型接合体およびアカタラセミア異型接合体は比較的正常に近いバンドを示した．このことから，アカタラセミアと正常マウスの赤血球カタラーゼ分子の等電点には差があり，よって両者の蛋白質の構造に差異があることが認められた．

B66. Introduction of Human γ_1-Immunoglobulin Gene into Mouse Feritized Eggs: Ken-ichi YAMAMURA,[1] Hitoshi KIKUTANI,[2] Naoki TAKAHASHI,[3] Shizuo AKIRA[1], Tetsuya TAGA[2], Kazuhiko KAWAI,[1] Kenichiro FUKUCHI,[1] Yuichi KUMAHARA,[1] Tasuku HONJO,[3] Tadamitsu KISHIMOTO[2] ([1]Dept. Med. Geriatr., [2]Cent. Cell Engineer., [3]Dept. Geriatr., Osaka Univ., Osaka)

Ch4A-VCE-γ_1, which we used includes a EcoRI and BglII fragment of PBR 322-CESS V (CE-1) containing VDJ region and a EcoRI and BamHI fragment of Ch4-HIgγ_1-10 containing γ_1 constant region. About 200 copies of Ch4A-VCE-γ_1 genes were introduced into mouse fertilized eggs. Of 489 eggs injected with these genes, 319 survived and these were transferred to oviducts of foster mothers. Thirty-eight were born and were screened for the presence of human γ_1-immunoglobulin genes by Southern blot hybridization to total DNA extracted from liver biopsy samples using probes for the human γ_1-immunoglobulin gene. Five of these 38 mice had integrated human γ_1-immunoglobulin sequences. Estimated copy numbers in each mouse were 20, 15, 4, 1, and 20, respectively. Majority of the human γ_1 copies in each mouse have not undergone any deletions or rearrangements as judged from Southern blotting pattern by several restriction enzymes. Human γ_1 gene was present in several different tissues. All the mice tested so far transmit the human γ_1 gene to a fraction of their offsping in an autosomal dominant manner. Spleen cells from Ch4A-VCE-γ_1 positive mice were analysed for immunoglobulin production either by reverse plaque assay or by immunofluorescence staining of cytoplasmic immunoglobulin. Secretion and production of human γ_1 chain could not be detected in these mice. The presence of human γ_1 immunoglobulin gene did not appear to have any effect on the expression of endogenous mouse immunoglobulin genes. Northern blooting analysis is now under progress.

C1. Critical Role of Monosomy for the Segment 3p25.3→p26.2 in 3p-Syndrome: Report of a Case with an Interstitial Deletion of 3p: Kiyoshi KIKKAWA, Kuniaki IYODA, Kouji NARAHARA, Shunsuke KIMURA and Hiroshi KIMOTO (Dept. Pediatr., Okayama Univ., Okayama).

The proband, a 10-month-old female infant, was born after 39 weeks of gestation to a 31-year-old mother and a 33-year-old father. The birth weight was 2,435 g and the height 42 cm. At 10 months of age, the infant was admitted because of psychomotor retardation and failure to thrive. Physical examination revealed that the weight was 5,985 g and the height 65.4 cm (both figures less than 1 percentile). She was found to have following abnormalities: microcephaly, flat occiput, anterior low hair line, triangular facies, synophrys, blepharoptosis, short palpebral fissures, upward slanting of eye slits, epicanthic fold, ear malformations, long philtrum, anteverted nostrils, carp shaped mouth, thin lip, high arched palate, micrognathia, brachydactyly, rocker bottom feet, and rectus diastasis. Study of auditory brainstem evoked response revealed severe degree of bilateral sensorineural deafness. Dermatoglyphics of the proband included right simian line, bilateral high axial triradii (t″), digital patterns consisting of UL A UL UL UL in the right palm and UL RL RL UL UL in the left palm, and low total ridge count (58). Cytogenetic studies using high resolution G- and R-banding techniques showed the karyotype of 46, XX,del(3)(p25.1;p26.2). The parents had normal chromosomes. Seven cases with a deletion of the short arm of chromosome 3 have been so far reported. Merrild *et al.* (1981) have claimed that 3p− has a syndrome characterized by severe psychomotor and growth retardation, a peculiar facies especially with triangular appearance and blepharoptosis, and anomalies of the extremities. The deleted segments in the 7 reported cases were p25→pter (6 cases) or p25.3→pter (1 case). The present case, in which deletion involved the segment p25.1→p26.2, showed a similar phenotype to that of the 3p− syndrome. Together with this, the lack of phenotypic features of the 3p− syndrome in a case with r(3)(p26.2q29) (Narahara *et al.*, 1981, unpublished) suggested that the loss of the segment 3p25.3→p26.2 is responsible for the 3p− syndrome.

C2. inv(9)(p22;q22), インスリン依存性糖尿病を伴った選択的 IgA 欠損症の1女児例：大塚武司・富沢修一・嶋倉泰裕・高野健一郎・大場正己・堺　薫（新潟大・医・小児），**太田　裕**（新潟県立がんセンター・小児）．**A Case of Selective IgA Deficiency with Insuline Dependent Diabetes Mellitus and inv(9)(p22;q22):** T. OHTSUKA, S. TOMIZAWA, Y. SHIMAKURA, K. TAKANO, M. OHBA, K. SAKAI (Dept. Pediatr., Niigata Univ., Niigata) and Y. OHTA (Dept. Pediatr., Niigata Cancer Cent. Hosp., Niigata)

IgA 欠損症には種々の疾患が合併することが知られており，染色体との関連では 18 番染色体の異常を伴う例が報告されている．今回，私たちは選択的 IgA 欠損症にインスリン依存性糖尿病

(IDDM) を合併し，inv(9)(p22;q22) の染色体異常を有した 11 歳女児例を経験した．症例は 7 歳時に多飲多尿で発症し IDDM と診断，以後インスリン療法を継続している．家族歴，既往歴，身体所見には異常なく，小奇形もない．免疫学的検索では血清 IgA, 418 μg/dl, 唾液分泌型 IgA 280 μg/dl, 唾液中 secretory component は正常で，7S, 11S IgA の両者の欠損を認め，リンパ球培養での IgA 産生能の検索では，患児の T cell 系には異常なく，B cell の IgA 産生形質細胞への分化，成熟過程の異常が示唆された．HLA では患児は本邦の IDDM に出現頻度の高い BW54 を有していた．G バンド，C バンドによる染色体検査での核型は 46,XX,inv(9)(p22;q22) であり，家系内では母親が同様の核型を有していたが，IgA 欠損症，IDDM ではなかった．IgA 欠損症と inv(9)(p22;q22) との関連は，他に同様の症例の報告もなく，現在のところ不明である．

C3. Double Minute Chromosomes (DMs) in a Case of Acute Myeloblastic Leukemia and the Cell Line Derived from the Patient: Yutaka OHTA (Dept. Pediatr., Niigata Cancer Cent. Hosp., Niigata) **and Kenji KISHI** (Dept. Intern. Med., Niigata Univ., Niigata)

In this report we present the cytogenetic findings from the patient with AML and the cell line, derived from the patient, which contain DMs and an aberration involving the short arm of chromosome 1 which is possibly homogeneously staining region (HSR).

The patient, aged 28 years, had a histroy of non-Hodgkin lymphoma. His disease had been clinically diagnosed as stage I, and he had undergone radiotherapy (5,600 R) in Jan. 1980. He was admitted to our hospital on Sep. 16 1981 because of lymphadenopathy and leukocytosis. He was diagnosed as AML (M2). Bone marrow samples were processed for chromosome analysis by a direct technique. In the first sample, out of 30 metaphase plates, 11 (36.6%) revealed DMs. In the second sample, out of 13 metaphase plates, 5 (38.5%) revealed DMs. The number of DMs varied between one and 18. Variation in the size of the DMs was observed. G-banding karyotype was 45,X,−Y,i(17q),del(1)(p32?), −22,+mar. The KY821 cell line was derived from liquor of the patient with CNS leukemia on Jan. 21 1982. The KY821 cell line had numerous DMs which number ranged 1–58 per cell and more than 84% of the cells contained DMs. The cell line had a modal number of 78, ranging from 60 to 88 chromosomes, and structural aberrations containing i(17q) and the distal region of 1P which was normally weakly banded.

On Oct. 5 1983 the number of DMs decreased.

C4. A Partial 10p Monosomy: Naoki NOMOTO, Akira YOSHIDA, Yuri MIYANOMAE (Dept. Pediatr., Kyoto City Child Welfare Cent., Kyoto), **Osamu NAGAUCHI** (Dept. Clin. Lab., Kyoto City Hosp., Kyoto) **and Haruyasu IKUTA** (Dept. Pediatr., Kyoto Ist Red Cross Hosp., Kyoto)

A 18 month old female with a few peculiar features, and mental and developmental retardation was reported. Her clinical features included; normal birth weight, downward slanted palpebral fissures, inner epicanthal folds, blepharophimosis, large mouth, low set ears, short commissura labiotrum posterior. She had no malformation of the heart. Computed tomography of the head revealed moderate frontal brain atrophy. Her weight was 9,200 g, length 78.4 cm, head circumference 48.4 cm, and chest circumference 46.4 cm. The proband was the product of a full term pregnancy born to a 25 year old woman (gravida I para O). Her father's ages was 24. She weighed 3,120 g and her head circumference was 35.6 cm at birth. During her neonate and early infant period she had severe jaundice, convulsion due to hypocalcemia, and failure to thrive. Her head was held by 6 months. She sat alone by 11 months, and now stands with support. Developmental ages was 8 months at 18 month old. Dermatoglyphic study showed palmar axial triradii in the t′ position, both simian lines, all whorles in fingertip patterns bilaterally, and L^d in the hallucal areas bilaterally. Using our modified G-banding method and Q-banding method, the proband's karyotype was *de novo* 46,XX,del(10)(p13), and parent's karyotype was normal.

C5. Another Retinoblastoma Patient of 13q14 Deletion Mosaicism with Mental Retardation and Muscular Hypotonia: Tomiko MOTEGI, Akira INOUE, Hiroko KADOWAKI, Makiko KAGA, Yukishige YANAGAWA (Dept. Pediatr., Tokyo Univ. Branch Hosp.), **Kazumi AKAZAWA, Mari KOMATSU and Kensei MINODA** (Dept. Ophthalmol., Tokyo Univ. Branch Hops., Tokyo)

In 1981 and 1982 Motegi reported five cases with 13q14 deletion mosaicism in lymphocytes among 66 retinoblastoma patients. All these five patients with 13q14 deletion mosaicism had normal somatic growth and normal mental development. We recently experienced a patient of a 13q14 deletion mosaicism in lymphocytes who had muscular hypotonia and mental retardation (D.Q. 49 at 13 months of age) besides the sporadic bilateral retinoblastoma. The proband, a 4-month-old boy (patient no. 67), was referred to the Department of Ophthalmology because of therapy to bilateral retinoblastoma. He is the first child of unrelated healthy parents. There was no family history of other anomalies. The pregnancy and delivery were uneventful. The mother was 25 and the father 28 years old at the time of the proband's birth. Birthweight was 3,250 g and length 48 cm. At two months of age, nystagmus and leukocoria in the left eye were noted. He

consulted another eye clinic at 3 months of age when the right leukocoria subsequently occurred. The ophthalmoscopy showed a solid mass of the size of 10 disc diameters associated with localized detachment of retina in the right eye and the total detachment of retina accompanied by corneal opacity in the left eye. Both ultrasonography and computerized tomography revealed bilateral retinoblastoma. He is a floppy infant with somewhat peculiar face including hairy forehead, slightly upwards-slanting palpebral fissures, broad nasal bridge, a large mouth with a thin upper lip, a long philtrum, bulbous tip of the nose and large pinnae with unusual pitting. His skull has a deformed configuration with the right parietal bossing.

The first blood of this patient in May, 1982 showed that the proportion of 13q14 deleted cells was 32% (123 cells examined using high resolution G-banding techniques), while the second blood drawn in Feb., 1983 showed that the 13q14 deletion cells made up only 10% of 163 cells examined using both GAG- and RBG-techniques.

C6. 16q− Syndrome in Association with a Balanced X;16 Translocation and Selective Inactivation of the Derivative X Chromosome: Y. FUKUSHIMA, N. KATSUMATA and Y. KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

Case report: The patient, a 2y 0m female, was born at 39w after an uncomplicated pregnancy to unrelated healthy 31y mother and 33y father. Birth weight was 3,600 g. She had growth retardation and severe mental retardation. Her facies was characterized by frontal bossing, up-ward slanting of short palpebral fissures, epicanthus, hypertelorism, skin tag on the nasal tip, anteverted nostrils, wide-spread nasal alae, median cleft lip, retrognathia, malformed ears and high-arched palate. She suffered from congenital heart disease (VSD) and ophthalmological abnormalities including microphthalmia, persistent pupillary membrane, corneal leukoma, heterochromia and internal strabismus. Her DQ (MCC) was estimated at 29. Dermatoglyphics was abnormal: distal axial triradius t′(bil), additional digital triradius d′(bil), decreased TFRC(76) and fibular arch hallucal pattern(lt). *Cytogenetic investigations:* The karyotype of the patient was 46,X,t(X;16)(q28;q11.2) on the basis of high-resolution G-banding with the 550-band stage. The parents were chromosomally normal. In X-replication studies using the BrdU-AO technique, the derivative X chromosome was constantly late replicating in all of 41 cells examined. *Comment:* Inactivation of the segment of 16q translocated on Xq seemed to be the most plausible explanation of multiple congenital anomaly and mental retardation in the present case. Four cases with 16q− have been published. Among them, 3 cases with the deletion of 16q21 band were very similar to each other. Though the present case shared some features with the previous cases, the majority of her phenotype were different from them. This

variation may be due to the difference of the breaking points and that in quality of chromosomal abnormality, *i.e.*, simple deletion or inactivation. There are many reports of balanced X; autosome translocations. In almost all cases, the normal X chromosome is constantly inactivated. But, in some exceptional cases the derivative X chromosome is inactivated and such cases are affected with various kinds of congenital diseases. The role of inactivation of X chromosome in balanced X; autosome translocations has not been clarified.

C7. Pseudo-triploidy (染色体数 69) の小児 AMMoL の 1 例：月野隆一・大森啓充・飯塚忠史・樋口隆造・小池通夫 (和歌山医大・小児). **An Infant Case of AMMoL with Pseudo-Triploidy (Chromosome Number 69):** R. TSUKINO, H. OHMORI, T. IIZUKA, R. HIGUCHI and M. KOIKE (Dept. Pediatr., Wakayama Med. Coll., Wakayama)

[研究目的] 小児白血病の重症度判定に染色体が指標となり得るか，約 50 例について追跡中である．今回 high-risk 群と思われる小児 AMMoL で発症時 pseudo-triploidy，末期に染色体数 47 のクローンのみになった 1 例を報告する．[方法] 症例 K・T 1 歳 11 か月男子．染色体分析は発症時骨髄，末期には末血を用い 24 時間培養法によった．G, C-バンド施行．[結果] 発症時骨髄細胞には骨髄単球様，FAB 分類 M4 の芽球 30%，染色体数 69 がみられた．各種抗癌剤使用後，骨髄は完全に dry tap となった．その後，増殖したのは骨髄芽球様 M1，染色体数 47 の細胞ばかりであった．全経過 17 か月で死亡した．[考察] pseudo-triploid の生成機序は，正常 diploid set＋異常 haploid set により，腫瘍の性質は異常 haploid set (予後不良群) を反映していると推定される．末期に出現した芽球は，FAB 分類 M1，染色体数 47 と発症時と全く共通性を示さず，15 種類に及ぶ抗癌剤による secondary tumor の可能性が強い．

C8. A Case of 48,XYY,+21: Kazushi NOMURA, Hideki TERAMOTO, Shinichiro TANAKA, Kozo OHAMA, Atsushi FUJIWARA (Dept. Obst. Gynec., Hiroshima Univ., Hiroshima) **and Koji SUNAHORI** (Dept. Obst. Gynec., Chugoku Rosai Hosp., Kure)

The patient was a 5-month-boy born as the first child to the parents, 19-year-old mother and 25-year-old father, at 39 weeks of gestation. He weighed 3,778 g at birth and main clinical findings were as follows: muscular hypotonia, dried skin, joint hyperflexibility, oblique eye fissures, epicanthic eye-folds, left ear deformity, flatness of nasal bridge, protruding tongue, short neck, loose skin of neck, broad and stumpy hands and feet, left short 5th finger, left incurved 5th finger and single crease on left 5th finger. Chromosome analysis by the G-, Q-, and R-banding techniques on peripheral blood lymphocytes revealed a karyotype of 48,XYY,+21. To investigate the origin of an additional no. 21 chromo-

some in the patient, fluorescent heteromorphism of no. 21 chromosomes of the patient and parents were examined using R and Q sequential staining method.

Two of the patient's no. 21 chromosomes showed homozygous pattern which originated from one of the two homologous chromosomes of the father. This finding suggests the following mechanisms as the possible cause yielding the abnormal chromosome constitution of the patient. (1) Nondisjunction of no. 21 and Y chromosomes at the second meiotic division in spermatogenesis. (2) Nondisjunction of no. 21 and Y chromosomes at the first cleavage division with death of a 21-monosomic cell line. (3) Nondisjunction of Y chromosome at the second meiotic division and nondisjunction of no. 21 chromosome (paternal origin) at the first cleavage division with death of a 21-monosomic cell line. Of 10 cases with a karyotype of 48,XYY,+21 reported in the literature, only one by Schmidt *et al.* (1978) was analyzed for the origin of the additional chromosomes and concluded to be due to second meiotic error in spermatogenesis.

C9. 各種の患者集団における異形染色体の調査（予報）：山田清美・田近令子・有田直子・神野一郎（国立病院医療センター・臨床研・遺伝）. **Survey of Q- and C-Variant Chromosomes in Various Patient Populations (Preliminary Report):** Kiyomi YAMADA, Reiko TAJIKA, Naoko ARITA and Ichiro JINNO (Natl. Med. Cent. Hosp., Tokyo)

[目的] Q および C バンド染色法によって染め出される染色体性の異質染色質は個体によって大きな量的変異がみられるが，この遺伝的形質が個体の表現型に全く影響をあたえないものなのかどうかを検討したい．そのため，これらの異形染色体の種類と出現頻度を正常者および各種の患者集団で調査し比較することを試みた．[方法] Q バンドはキナクリン染色法，C バンドは DA-DAPI 染色法を用いた．変異バンドの大きさの分類基準は，Q バンドは山田・長谷川 (1978) の方法を，C バンドは Pantil·Lubs (1977) の方法を用いた．[結果] 各集団で 200 例を目標として分析中であり結論を得ていないが，つぎの 7 つの集団を対象としている．括弧内は分析済人数．白血病患者群 (40)，3 回以上の反復流産歴をもつ夫婦群 (112)，初期に自然流産した胎児群 (11)，XYY 型クラインフェルター症患者群 (50)，21 トリソミー型ダウン症患者群 (188)，精薄者群 (165)，および正常者群 (197)．種々の方法論的問題点を述べ，2, 3 の注目している点(Y 染色体の長さ，逆位の頻度など)に言及した．

C10. 検査センターにおける染色体異常の出現頻度の推移とその分類：酒井恵子・伊東千春・二瓶文雄・横沢拓郎 (Special Ref. Lab.). **Chronological Changes in the Frequency of Chromosome Abnormalities in Specimens Analysed in a Laboratory:** Keiko SAKAI *et al.* (Special Ref. Lab., Tokyo)

当センターにおける染色体異常の統計処理分析（第 3 報）がまとまったので，ここに報告する．1981 年 7 月より 1983 年 5 月までに当社に依頼された検体の中で，20 細胞以上分析可能であった 15,117 検体について検査を実施した．方法としては末梢血液培養法，および各種分染法 (G, C, Q, R) を用いた．総検査数 15,117 検体中，染色体異常が確認されたのは 2,532 検体で，16.76% であ

った (1979 年, 3,829 検体中, 949 検体, 24.79%, 1980 年, 7,788 検体中, 1,783 検体, 22.89%). また, それを性染色体異常と常染色体異常に分類した場合, 前者は 3.26% (1979 年, 5.34%, 1980 年, 5.65%), 後者は 13.5% (1979 年, 19.45%, 1980 年, 17.24%) であり, 検査総数に対する異常率は年々低下の傾向にある. しかし, 異常者数については明らかに増加している. これは染色体検査自体が, ルーチン検査として一般的に普及してきたために異常率が低下しているものと考えられる.

C11. Karyotype Analysis in Many Cells from Chronic Myelocytic Leukemia (CML) Reveals Clones with Additional Ph^1 in Early Chronic Phase. **Kimio TANAKA,[1] Nanao KAMADA,[2] Nobuo OGUMA,[2] Yasuo TAKIMOTO,[2] Atsushi KURAMOTO[2] and Takeshi OHKITA[1]** ([1]Dept. Hematol., [2]Dept. Intern. Med., RINMB, Hiroshima Univ., Hiroshima)

Chronic myelocytic leukemia (CML) is a disease of clonal origin in which it has been suggested that the leukemic cells arise from a single Ph^1 positive cell. In more than 75% of the patients examined, additional karyotype abnormalities have been reported in the terminal acute phase of the disease. While studying the clonal evolution of karyotype changes in CML, we detected, by analysing many cells, the presence of clones with additional Ph^1 chromosome even in the early chronic phase of the disease. Bone marrow cells from 16 patients with CML were cultured for 24 hr and metaphase preparations were G-banded. More than 100 cells were karyotyped per each case. The following observations were recorded: 1) In all patients examined, an additional Ph^1 positive clone was noted in 1.3~12.4% of cells examined, in the early chronic phase. 2) Cells with additional Ph^1, also exhibited either numerical aberrations involving trisomy for #9, 15, 18 or unbalanced rearrangements involving #1, 4, 5 or 8. 3) A small proportion of cells in early chronic phase presented +8, 2Ph^1, or +17. This kind of aberration has been reported previously mostly in acute phase of CML. 4) Comparative karyotype analysis of early chronic and acute phase of 5 out of 16 patients who entered into acute stage, showed a rapid proliferation of clones with additional Ph^1 in only 2 patients, while in other 3, new clones appeared. These results suggest that analysis of many cells in early chronic phase may reveal subclones with additional Ph^1. Furthermore, data presented here may indicate that of all the chromosomal rearrangements seen in early chronic phase, only a few get selected and established in the terminal acute phase.

C12. Spontaneous Structural Aberrations of Lymphocyte Chromosomes in the Hiroshima F_1 Population: Akio A. AWA, Yoshiaki KODAMA and Takeshi ABE (RERF, Hiroshima)

Structural aberrations of chromosomes have been considered as one of the most sensitive indicators for the evaluation of the effects of physical, chemical and biological mutagens on man, since the yield of chromosome aberrations is determined by the dose of mutagens administered. It is thus important to accumulate the data on the type and frequency of spontaneously occurring chromosome aberrations in somatic cells of persons in the general human population. To privide basic information, the present observation was performed to determine the frequency of spontaneous somatic chromosome aberrations in approximately 6,000 children, born to A-bomb survivors and their controls in Hiroshima, who are considered not to have had any history of significant exposure to ionizing radiation prior to this survey. Of the 64,120 cells analyzed, dicentrics were noted with a frequency of 1.87×10^{-3}. This value appears somewhat higher than that obtained by other laboratories, showing a range between 0.2 and 1.3×10^{-3}. There were 18 persons, 12 males and 6 females, each containing a cell with multiple complex chromosome aberrations of unidentifiable nature, designated as MA cells. Biological implication of MA cells remains unclear, but they are presumed to be of T-cell origin, normally responding to PHA and thus entering into their *in vitro* mitosis. The results of preliminary experiments indicated that neither low temperature storage of whole blood prior to the initiation of culture nor the age of donors was responsible for the cause of MA.

C13. Structural Chromosome Aberrations in the Hiroshima F_1 Population: Yoshiaki KODAMA, Masashi HIRAMOTO, Kazumi TANABE, Junso NARUTO and Akio A. AWA (RERF, Hiroshima)

Somatic chromosomes of the children of atomic-bomb survivors (F_1) have been screened as a part of the RERF program searching for genetic effects of A-bomb irradiation on the Hiroshima and Nagasaki population. Chromosome analysis has been completed to date for 9,305 children (4,476 males and 4,829 females) in Hiroshima. Of these, 60 were found to have an abnormal chromosome constitution; 32 with autosomal anomalies and 28 with sex chromosome anomalies. The frequencies of abnormal individuals are similar to those of newborn infants obtained from neonatal cytogenetic surveys in several western countries. For the abnormal cases so far detected, the break points and the origin of abnormal chromosomes were determined by the conventional stain, G-, Q-, C- and N-banding techniques for 32 cases with structural rearrangements of both autosomes and sex chromosomes; 11 with reciprocal translocation, 10 with Robertsonian translocation, 9 with pericentric inver-

sion, 2 with an extra minute chromosome, one mosaic case with terminal deletion and one with a ring chromosome. A high resolution banding technique has also been applied to some cases sampled recently for the exact identification of the break sites.

C14. The Effect of Prolonged Treatment with Colcemid on Human Lymphocyte Chromosomes *in vitro*: Kazumi TANABE, Shozo IIDA, Yoshiaki KODAMA, Mimako NAKANO, Hachiro SHIMBA, Takeshi ABE and Akio A. AWA (RERF, Hiroshima)

In order to analyze the cells with radiation-induced chromosome aberrations in blood lymphocytes from aged A-bomb survivors for 48 hr of culture, it is essential to obtain a maximum number of well-spread metaphases from as small amount of blood as possible. It is also desirable that all the observable metaphases should be in their first *in vitro* cell division. To fulfill the above requirements, preliminary tests were conducted to accumulate efficiently metaphases by prolonged treatment of human lymphocytes in culture with a low concentration of colcemid. The rate of metaphases in the second *in vitro* mitosis to those in the first mitosis was measured by the presence of BrdU (5 μg/ml) in cultures for sister chromatid differential staining. The durations of colcemid treatment at a final concentration of 0.2 μg/ml were 2 h, 12 h, 24 h, and 48 h (or throughout incubation period). Mitotic indices in samples from 48-colcemid-treatment showed 2.5 times higher than those from 2 h-treatment. About a half of metaphases derived from 48 h-treatment was judged as eligible for detailed aberration analysis. The rates of metaphases in the second *in vitro* mitosis were less than 30% for 2 h-treatment, 0.2% for 24 h, and 0.05% for 48 h, respectively. Among various ranges of colcemid concentrations so far tested, the most optimal value was found to lie between 0.2 and 0.4 μg/ml, by showing the highest mitotic index and by the absence of cells in the second mitosis. A preliminary application of the continuous colcemid treatment of human lymphocytes *in vitro* from several A-bomb survivors showed that this technique is extremely feasible for the accumulation of metaphases with reasonably high quality arrested at their first *in vitro* cell cycle.

C15. SCE Frequencies in Cultured Human Lymphocytes—Analysis in Metaphases at the Third *in vitro* Mitosis: Mimako NAKANO and Akio A. AWA (RERF, Hiroshima)

In our previous observations, there was a wide variation in the base-line SCE frequencies among individuals. It remains to be demonstrated whether this phenomenon may be due to aging or may reflect the exposure of individuals to potential SCE-inducing substances,

such as smoking. In order to answer this question, SCEs were scored in metaphases at the third *in vitro* mitosis, harvested at 76 h of incubation, in terms of i) SCEs occurring in the first plus second cell cycle ($SCE_{(1+2)}$), ii) SCEs occurring in the third cell cycle (SCE_3), and iii) SCEs observed at the site of centromere (cenSCE). Results from 21 females and 19 males indicated that a ratio of $SCE_{(1+2)}$ to SCE_3 ranged from 2.8 to 6.4 with an average of 3.9. The mean of SCE_3 showed 2.1 and 2.3 for females and males, respectively. Although there was no increase with increasing age in the $SCE_{(1+2)}$ frequencies among females, there seemed to be a positive correlation of the $SCE_{(1+2)}$ frequencies with age in the males. This confirmed the previous results obtained from metaphases at the second *in vitro* mitosis. There was no effect of aging on the SCE_3 and cenSCE frequencies in both sexes. In order to deliberate the age-related increase in the $SCE_{(1+2)}$ frequencies observed only in males, a retrospective survey was examined for personal smoking habits. Mean frequency of $SCE_{(1+2)}$ from 5 smokers and 3 ex-smokers were 8.40 and 7.29, respectively, while 10 non-smokers (control) showed 7.01. On the other hand, the frequencies of both cenSCE and SCE_3 in the smokers and ex-smokers were almost the same as control level. Exceptionally high frequencies of $SCE_{(1+2)}$ were observed in 2 cases (12.6 and 14.4). The reason for unusually high frequencies remains unknown. It is possible to interpret that positive but apparent relationship between $SCE_{(1+2)}$ frequencies and aging may be influenced by potential exposures of individuals to genotoxic substances.

D1. Studies on the Distribution of Blood Group Substances in the A_{int} Red Cell Membranes: Shin YAZAWA, Tamiko NAKAJIMA and Ken Furukawa (Dept. Legal Med., Gunma Univ., Maebashi)

The blood group A_1, A_{int} and A_2 subgroups are characterized by a continuous spectrum of the agglutinability against anti-A serum, anti-A_1 Dolichos lectin and anti-H eel serum. The A-enzyme activity in the red cell membranes was detected to be linearly related to the A-activity of the red cells. The H-sites in the membranes of A subgroups and group O were glycosylated with UDP-GalNAc[^{3}H] and purified plasma A_1-enzyme. The contents of H-sites in the A_1, A_{int} and A_2 membranes were 16.2%, 40.0% and 75.0%, respectively, of that in the O membranes. The labeled membranes from group O, A_2 and A_{int}, thus produced, were analyzed by SDS-polyacrylamide gel electrophoresis. It was demonstrated that the labeled products of the O and A_2 membranes were located mainly in the macroglycolipid component, but that those of the A_{int} membranes were located in the region between M.W. 50,000–90,000. Analysis of the labeled products by isoelectric focusing (pH 4.2–8.2) showed that the O and A_2 membranes had three radioactive peaks between pH 6.0 and 7.2, whereas the A_{int} membranes had smaller ones between them and had a broad peak in the alkaline region. These results suggested that qualitative differences of H and A substances exist in the A_{int} membranes, independently of their intermediate characterization. The amount of unglycosylated H-sites in the A_{int} B membranes was small as in the A_1B membranes. After the blood group B substance in the membranes was decomposed with $\alpha 1 \rightarrow 3$ galactosidase from *Cl. sporogenes* Maebashi, the amount of GalNAc incorporated into the membranes was increased.

D2. Studies on Salivary Protein Polymorphisms. Electrophoretic Analyses of Human and Macaque Monkey Salivas: Goichi ISHIMOTO, Tsukasa INAGAKI, and Hideaki UDA (Dept. Legal Med., Mie Univ., Tsu)

Four human parotid salivary proteins were phenotyped in 460 individuals, consisting of students and lab. personnels, by isoelectric focusing at pH 3–6 and alkaline polyacrylamide gels. The gene frequencies ($Pr^1=0.76\pm0.01$ $Pr^2=0.24\pm0.01$, $Pa^+=0.21\pm0.01$ $Pa^-=0.79\pm0.01$, and $Db^+=0.04\pm0.01$ $Db^-=0.96\pm0.01$) obtained for Pr (proline-rich), Pa (acidic) and Db (double-band) are very similar to those of the previous reports by Ikemoto *et al.* (1977, 1979), and those ($PIF^+=0.70\pm0.02$ and $PIF^-=0.30\pm0.02$) of PIF (isoelectric focusing) figured for Japanese. As already pointed out by Azen and Denniston (1978, 1981), strong association of the phenotypes, Pr 2 and Pa+ and also Pr 2 and PIF−, is evident in the population. The haplotype frequencies ($Pr^1Pa^+=0.013$, $Pr^1Pa^-=0.748$, $Pr^2Pa^+=0.194$ and $Pr^2Pa^-=0.045$) are presented especially for practical purposes. Animal

salivas were collected from 15 Macaca fuscata monkeys with pilocarpine stimulation, and concentrated samples were electrophoresed to examine the saliva protein variation. As electrophoretic patterns were quitely different between humans and monkeys, the estimation of the monkey bands corresponding to human Pr, Pa, Db *etc.* was almost impossible on protein-stained gels. By dimethoxybenzidine stain, monkey saliva shows two different peroxidase-active proteins, one broad zone in the middle of the gel and another sharp band(s) far at the anodal edge of the alkaline polyacrylamide gel. Although the former proteins are invariably found in all samples, the latter clearly shows intra-species differences, which comprise 3 phenotypes with one or two main bands, suggesting the existence of salivary protein polymorphism in macaque monkeys.

D3. Genetic Polymorphism of Human Parotid Salivary Proteins and Isozyme (Pa, Pb, Pr, Db, Pm, Ph, Pn and s-AcP) and Whole Salivary Amylase Isozyme among Japanese Population in Tochigi Prefecture: Shigenori IKEMOTO (Lab. Hum. Biol., Jichi Med. Sch., Tochigi)

Phenotype and gene frequencies of parotid salivary proteins, Pa, Pb, Pr, Db, Pm, Ph, Pn and parotid isozyme, s-AcP and whole salivary amylase isozyme were studied among Japanese population in Tochigi prefecture. The Pa system was examined in 324 individuals and the gene frequency obtained was 0.2143 for Pa^+ and 0.7857 for Pa^-. In the Pb system, all 324 samples showed Pb 1-1 type and Pb^3 allelic gene was not found. The Pr, Db, Pm, Ph and Pn systems were examined in 333 individuals and the gene frequencies were 0.7597 for Pr^1, 0.2403 for Pr^2, 0.0534 for Db^+, 0.9466 for Db^-, 0.4138 for Pm^+, 0.5862 for Pm^-, 0.0288 for Ph^+, 0.9712 for Ph^-, 0.1247 for Pn^+, and 0.8753 for Pn^-. The Amy_1 phenotypes were 50 individuals and the gene frequency of $Amy_1{}^v$ was 0.010. The s-AcP phenotypes were examined in 204 individuals and the estimated gene frequency was 0.2304 for $s\text{-}AcP^1$ and 0.7696 for $s\text{-}AcP^2$. The s-AcP system is a new genetic marker discovered by the authors and found in the parotid salivary isozyme.

D4. Alpha-1-Antitrypsin (PI) Polymorphism in the Japanese: Confirmation of PI*M4 and Description of New PI Variants: Isao YUASA (Dept. Legal Med., Tottori Univ. Sch. Med., Yonago), **Kazuyuki SUENAGA** (Yamaguchi Red Cross Hosp., Yamaguchi), **Yasuhiro GOTOH** (Shimane Red Cross Blood Cent., Matsue) **and Keiichi ITO** (Yamaguchi Red Cross Blood Cent., Yamaguchi)

Application of isoelectric focusing (IEF) to PI phenotyping has permitted the discovery of four PI subtypes and a number of PI variants. In order to calculate the gene frequency of *PI*M4* in the Japanese, the PI phenotyping by separator IEF was performed

on a total of 1,000 serum samples from two local Japanese populations. Some serum samples were submitted to acid starch gel electrophoresis and print immunofixation. The PI M3 and M4 bands were distinctly separated from the PI M1 bands. The PI M4 bands were slightly cathodal to PI M3 bands. A difference between the PI M3 and M4 was very small but distinct. These appearances correspond to the four-allele model for the inheritance of PI M subtypes. Only three examples were typed as PI M1M4. *PI*M4* was confirmed to be present in the Japanese but was extremely rare as well as PI variant alleles. The gene frequencies obtained are as follows: $PI^*M1=0.7065$, $PI^*M2=0.2390$, $PI^*M3=0.0480$, $PI^*M4=0.0015$ and PI^*variant$=0.0050$.

The following six variants were observed in this study. Variant 1 (E Matsue): this new variant run between E Lemberg and E Franklin. Variant 2 (E Tokyo): this variant was already observed in the Japanese. Variant 3 (N Nagato): this new variant was almost similar to N Adelaide. However, D TT-IAC treated N Nagato migrated between N Adelaide and N Hampton. DTT-IAC treatment was also effective in differentiating P Castoria from P Kyoto. Variant 4 (P Onomichi): this variant was located between P St Louis and R and was the same as P Fukuoka. Variant 5 (P Yasugi): this new variant had a mobility between P Clifton and P Leverkusen. This difference between P Yasugi and P Leverkusen was observed only in DTT-IAC treated samples. Variant 6 (Y Hagi): this new variant run between X and Y Toronto.

D5. Isoelectric Focusing Studies of Human Red Cell Esterase D in Japanese: Tohru ITOH and Itsuro NISHIGAKI (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Human redce ll esterase D (EsD) exhibits genetic polymorphism with three common phenotypes determined by two autosomal codominant alleles, EsD^1 and EsD^2. A large number of population data have clarified the allele distribution in various races and additional rare alleles have hitherto been detected: EsD^3, EsD^4, EsD^5, EsD^6, and a null allele EsD^0. Recently, it has been elucidated that the allele EsD^5 distributes with a polymorphic frequency in Caucasian populations. This allele could be easily discriminated from the allele EsD^2, using the isoelectric focusing (IEF) technique, but the IEF analysis has been so far done only in Caucasians and in small samples of Negroes. IEF investigations of EsD were therefore performed on Japanese samples. By means of starch gel electrophoresis, EsD phenotypes and allele frequencies were calculated in 1,823 samples, not different from those of other Japanese populations, while IEF analysis on same individuals revealed no presence of EsD^5 but the occurrence of a rare and hitherto unknown allele. Although we tentatively designate this allele EsD^7, isoenzyme products of which are very closely

located to those of EsD^1 in electrophoresis, this existed with a frequency suggestive of polymorphism ($EsD^7=0.008$). In quantitative analysis as to individuals with EsD^7, remarkably low values were found: $EsD^1/EsD^7=3.5$ and $EsD^2/EsD^7=1.5$. These results suggest that subtyping by IEF should be performed in chromosome mapping analysis, population genetic studies and even forensic medicine investigations such as paternity testing.

D6. Polymorphism and Rare Electrophoretic Variants of Glutamate-Pyruvate Transaminase (GPT) in Japanese Residing in Hiroshima and Nagasaki: Yasukazu KIMURA, Junko KANEKO, Norio TAKAHASHI, Kazuaki GORIKI, Mikio FUJITA, Chiyoko SATOH (RERF, Hiroshima) **and Ryuji HAZAWA** (RERF, Nagasaki)

With the aim of evaluating the possible genetic effects of A-bomb radiation at the protein level, a study is being made to detect electrophoretic variants of 30 blood proteins of children born to proximally exposed survivors (study group) and those of distally exposed survivors (control group). This report presents results on glutamate-pyruvate transaminase (GPT) in red cell hemolysate. Vertical starch gel electrophoresis and GPT staining were conducted according to the method of Chen and Giblett (1971)[1] on a total of 13,772 children, 7,122 in the study group and 6,650 in the control group. A slow variant with a mobility similar to that of GPT 6 was found in a child whose mother was exposed in Hiroshima (γ 3 rad, neutron 1 rad). Both parents showed GPT 1. Since no inconsistency was observed in the biological parentage by the examinations of blood, protein and HLA types, this variant detected in the child is assumed to have been induced by a fresh mutation. The frequency of *GPT*1* for the cities combined, 7,187 Hiroshima children and 6,585 Nagasaki children, is 0.592 which falls within the range of 0.535–0.624 reported by others for the Japanese. There is no significant difference in the frequency of *GPT*1* between Hiroshima and Nagasaki. Seven types of hereditary rare variants were encountered among 83 children, 75 migrating more anodally than the GPT 1 band and 8 migrating more cathodally. They are tentatively named 8 HR1, 8 NG1, 8 HR2, 9 HR1, 4 NG1, 7 HR1 and 6 NG1. A group of fast migrating variants with mobilities similar to that of 8 was divided into three types: 8 HR1, 8 HR2 and 8 NG1. 8 NG1 was found in 19 families in Nagasaki, but only in 6 in Hiroshima. The difference in the frequency of *GPT*8NG1* between Hiroshima and Nagasaki is highly significant ($p<0.01$).

1) Chen, S.H. and Giblett, E.R. 1971. *Science* **173**: 148.

D7. The Gene Frequencies of Glutathione-*S*-Transferase Isozymes in Japanese Population: Katsunori AKIYAMA and Kazue ABE (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Glutathione-*S*-transferase [EC 2.5.1.18] GST consists of the three different isozymes GST_1, GST_2 and GST_3 that were detected by starch gel electrophoresis and the enzyme specific staining procedures using liver extracts. Furthermore, the phenotypes of GST_1 were separated into GST_1^1, GST_1^{2-1}, GST_1^2 and GST_1^0. And the phenotypes of GST_2 were separated into GST_2^1, GST_2^{2-1} and GST_2^2. The electrophoretic patterns of GST_3 show the high-activity forms or low-activity forms individually in starch gel electrophoresis using human red blood cells. We present the data on the distribution of GST_1 and GST_2 phenotypes using liver extracts of a total 118 unrelated individuals in Japanese population, and GST isozymes using leukocyte extracts. The gene frequencies of GST_1^1, GST_1^2 and GST_1^0 were estimated at 0.2411, 0.0934 and 0.6655, respectively. And the gene frequencies of GST_2^1 and GST_2^2 were estimated as 0.7924 and 0.2076, respectively. It was shown that the investigation of GST isozymes using leukocyte extracts reveals the genetic polymorphism of GST_1 and the high-activity forms or the low-activity forms of GST_3. The gene frequencies of GST_1 and GST_2 in Japanese population were similar to those of the other races.

D8. Genetic Polymorphism of Mitochondrial Glutamate-Oxaloacetate Transaminase in Japanese: Tasuku TOYOMASU, Koichi SUZUKI, Naoki KAWAI and Kiyoshi MATSUI (Dept. Legal Med., Osaka Med. Sch., Osaka).

This report describes an existence of a genetic variant of m-GOT in Japanese population which was detected by the analysis of a number of sera with the recently devised electrophoretic procedure (Sakakibara *et al.*, 1983). Cellulose-acetate membrane electrophoresis was carried out followed by the GOT specific staining using cysteine sulfinate as substrate instead of aspartate. The variant pattern was confirmed by starch gel electrophoresis using white cells as sample and aspartate as substrate conventionally. Sixteen out of 1,860 samples showed triple banded pattern that was indistinguishable from mGOT2-1 on starch gel (Hackel *et al.*, 1972). Studies on two families confirmed the autosomal codomi nant inheritance of the variant.

D9. Computer Simulation of Polygenic System: Eiji INOUYE, Katsumi MITA (Inst. Develop. Res., Kasugai) **and Shigekazu TANAKA** (Dept. Engineer. Physics, Chubu Inst. Technol., Kasugai)

Statistical analysis of threshold characters has taken a normal distribution of liability for granted, but it is conditional on a large number (N) of contributing gene loci ($15<$

$2Np$) and a constant allele frequency (p), both of which do not hold for actual situation. Ignoring epistasis, taking independent loci, each with two alleles, and let N=3, 4, 6, 8, 12 and 16, p_N ($0 \leq p_N \leq 1$) and genotypic value (E) of homozygotes ($2A_{1,N}$ and $2A_{2,N}$; $0 \leq A_{1,N}, A_{2,N} \leq 1$) at Nth locus were generated at random. Genotypic value of heterozygotes was either (1) $A_{1,N}+A_{2,N}$ (semidominance model), (2) $2A_{1,N}$ (complete dominance model), (3) between $2A_{1,N}$ and $2A_{2,N}$ (incomplete dominance model), or (4) between -1 and $+3$ (overdominance model), and in models (3) and (4) the value was taken at random. For every type of genotype combinations $Y=\pi F$ (F is genotype frequency at each locus) was computed and plotted against fifteen classes of $X=\Sigma E$. Computations were repeated three times. Approximation to normal distribution was achieved for $8<N$ in semi- and overdominance models, and for $16<N$ in complete and incomplete dominance models. In smaller number of gene loci the distribution was either discrete or involving greater skewness and/or kurtosis, and should therefore involves a substantial error.

D10. A Case of a Kabuki Make-up Syndrome with Growth Hormone Deficiency: **Hiromu FUNAKI, Noriko NAKADA, Kazuyuki ISHITOBI, Takao SASAKI** (3rd Dept. Intern. Med., Tottori Univ.), **Atsushi IESHIMA** (Div. Child Neurol., Tottori Univ., Yonago), **Hiroaki HOSODA** (Dept. Pediatr., Hiroshima Pref. Hosp., Hiroshima)

Kabuki make-up syndrome is a new malformation syndrome reported by Niikawa, Kuroki *et al.* (1980). This syndrome is characterized by peculiar faces, dwarfism, mental retardation and abnormal dermatoglyphics. Causes of growth retardation remain unknown. This 5-year, 11-month-old boy was a first-born and the product of a 32-week-gestation breech delivery to unrelated parents. Maternal and paternal ages were 29 and 30, respectively, at the patient's birth. Birth weight was 2.3 kg. He showed susceptibility to upper respiratory infection and otitis media. On examination, he was 94.6 cm in height (-3.9σ) and 12.5 kg in weight. Bone age was 3 years. He has a peculiar face: long palpebral fissures, eversion of the lower eyelids, sparse eyebrows, heavy and long eyelashes and large ears. He has abnormal dermatoglyphics including bilateral simian lines, finger pads and absence of digital triradius c. There are high-arched palate, retention testis, scoliosis and absence of right 12th rib. His DQ was 58. The karyotypes of the patient and his mother showed normal. The results of endocrinological examination were as follows: GH deficiency was revealed by provocative tests including insulin, arginine, glucagon and L-dopa. PRL responses to TRH and sulpiride were also blunt, but secretions of TSH, LH, FSH and ACTH were normal. Oral glucose tolerance test gave a normal pattern. GH deficiency may play an important role in growth retardation in this patients.

D11. Abnormal Distribution of Argininosuccinate Synthetase in the Liver of Citrullinemic Patients with Socalled Quantitative Type of the Enzyme Abnormality: **Takeyori SAHEKI, Yukio YAGI, Mariko SASE and Yasushi IMAMURA** (Dept. Biochem., Kagoshima Univ., Kagoshima)

From the enzymatic and immunochemical analysis, we found two types of citrullinemia, qualitative and quantitative types of the enzyme abnormality in Japan. In the qualitative type, a decreased argininosuccinate synthetase (ASS) activity is found in the liver and the other organs, which is due to its abnormal kinetic properties, such as larger K_m values. On the contrary, a decreased ASS activity in the liver of the quantitative type is caused by a decrease in the amount of the enzyme protein which shows a normal kinetic properties. It is notable that ASS in the other organs of the quantitative type is normal in activity and kinetic properties. To answer the question as to whether the amount of the enzyme protein is decreased in the liver of quantitative type and whether the distribution of ASS in the louble is normal, we used immunohistochemical techniques to locate ASS in the liver of the citrullinemic patients. ASS was located in the cytosol of the hepatocytes of control liver, but not in the bile duct cells or endothelial cells, and distributed almost homogeneously in the lobule with slightly denser staining in the periportal region. We obtained two types of ASS distribution in the liver of quantitative-type citrullinemia; homogeneous distribution of ASS similar to that of control liver and heterogeneous distribution of ASS in which strongly stained hepatocytes make clusters among less stained hepatocytes like mosaic. As far as tested, we could find no such heterogeneous distribution of ASS in the hepatitis or cirrhotic liver, and no such heterogeneous distribution of arginase, aldolase or γ-glutamyltranspeptidase in the liver which shows the heterogeneous distribution of ASS. From these results, we suggest that the heterogeneous distribution of ASS is due to an abnormal gene expression of ASS in the individual hepatocytes.

D12. 複雑な心奇形を伴った胸腹結合体の 1 剖検例：喜友名琢也・楚南盛章・池田琢哉・平山清武（琉球大・小児），**松井克明**（琉球大・1 病理），**先成英一・早川国男**（宮崎医大・小児）. **An Autopsy Case of Thoraco-Abdominopagus Associated with Complicated Cardiac Anomalies:** Takuya KIYUNA, Moriaki SONAN, Takuya IKEDA, Kiyotake HIRAYAMA (Dept. Pediatr., Ryukyu Univ. Sch. Med.), Katsuaki MATSUI (1st Dept. Pathol., Ryukyu Univ. Sch. Med., Naha), Eiichi SENNARI and Kunio HAYAKAWA (Dept. Pediatr., Miyazaki Med. Sch., Miyazaki)

症例は女児，生後 4 日．母親は 25 歳，主婦．今回初回妊娠．近親結婚はなく，また家系に双胎あるいは奇形の発生を認めない．出産 1 年前に子宮筋腫摘出術を受けている．妊娠歴にも特記すべきことはない．13 週で腹部エコーによる双胎の診断を受け，38 週，帝王切開にて出産．仮死はなく生下時体重は 4,996 g．生後 4 日目に死亡し，剖検により以下の所見が確認された．患児は，胸骨上縁か

ら臍直上部まで癒合した二頭四脚四腕体の二重奇形であった．心臓は，Fritzsche の心奇形分類の III 型に相当し，単一心型を呈していた．心房には隔壁がなく単一．右側の両大血管には共に円錐が認められ，大動脈が閉鎖した肺動脈の右側に位置しており，右室様構造の単一心室より起始していた．左側の大血管は正常の位置関係で，左室様構造の心室より起始しており，右室と思われる Ludimental outlet chamber を認めた．上行大動脈は低形成で，大きな動脈管が開存していた．肺分葉は，右児の場合両側 3 葉，左児は右 3 葉左 5 葉であった．肝は癒合しており，門脈および下大静脈はおのおの 1 本ずつ認められた．脾は右児で欠損．臍帯は 1 本で，その中に 2 動脈 2 静脈を認めた．本症例のように無脾症を合併する胸結合体はしばしば認められ，本来 5 週頃形成されるはずの脾の発生に関して，5 週以前の因子が関与している可能性が示唆され，興味深く思われる．

D13. Autosomal Dominant Inheritance; A Pedigree of Cornelia de Lange Syndrome: **Hiroshi NAKAI, Keiya TADA** (Dept. Pediatr., Tohoku Univ.), **Touru KURASHIGE, Hidee FUJIMOTO** (Dept. Clin. Lab. Diagnosis, Tohoku Univ., Sendai), **Akemi ISHII and Seiko TAMAHASHI** (Dept. Pediatr., Saka Hosp., Shiogama)

A mother, her boy and her girl showed clinical features of Cornelia de Lange syndromes. The mother, 31-year-old, was born at the 8th month of normal pregnancy. Her birth weight was 1,200 g. She showed short stature, microcephaly, palpebral ptosis, hypertrichosis on the forehead, synophrys, small ala nasi, long philtrum, high arched palate, thin lip, low set ears, distal loop on the third interdigital area, absence of main line C and moderate mental deficiency. But she had no history of convulsions nor anticonvulsant medications. Her 3-year-old boy was born at the 38th week of pregnancy. His birth weight was 1,740 g. His clinical features were single transverse palmar creases, single flexion creases of the fifth fingers, clinodactylies, tapering fingers, cryptorchidism. He sat alone at his 10th month of age and began to walk at 2-year-old. He could not speek any words. The second child, a girl of 10 month, was born at the 39th week of pregnancy. Birth weight was 2,220 g. She had also hypertrichosis on the forehead, synophrys, high arched palate, and she could not sit alone yet. All of the family members were revealed no chromosomal abnormalities even by high resolution G-band methods. There was only one report of a pedigree on which this disorder had succeeded in more than two generations (Ptacek *et al.*, 1963). This family suggested an autosomal dominant inheritance for Cornelia de Lange syndrome.