日本人類遺伝学会第 29 回大会一般講演要旨

Abstracts of General Contribution, the 29th Annual Meeting of the Japan Society of Human Genetics

一般講演　General Contribution

I-1. Comparative Immunochemistry of Human Blood Group A Glycosyltransferases: **Hisao TAKIZAWA** (Natl. Res. Inst., Police Sci., Tokyo; Present address: Dept. Legal Med., Fac. Med., Toyama Med. Pharm. Univ., Toyama)

The purpose of this study is to examine the immunochemical properties of human blood group A glycosyltransferases. Enzymes were isolated from fresh urine of healthy men of each blood group. First, the immune serum produced by the injection of an A_1-enzyme-preparation into a blood group O′ rabbit inhibited not only A_1-enzyme action but also B-enzyme action. The serum absorbed with a B-enzyme-preparation still had an ability to inhibit the A_1-enzyme action and named A-enzyme-specific antiserum.[1,2] Second, qualitative differences between the A_1-gene-dependent and A_2-gene-dependent enzymes were demonstrated in the conversion of blood group O red cells into A and in the transfer of *N*-acetylgalactosamine-1-^{14}C. Third, the specific antiserum described above was cross reactive to A_2-enzyme. The data suggest a close resemblance between the structures of A_1- and A_2-enzymes.

1) Takizawa, H. and Iseki, S. 1982. *Proc. Jpn. Acad.* **58 (B)**: 226–228.
2) Takizawa, H. and Iseki, S. 1983. *Proc. Jpn. Acad.* **59 (B)**: 247–250.

I-2. ABO 遺伝子支配の型合成酵素と型物質の特異活性：宮崎生子・中島たみ子・小暮正久・古川　研（群馬大・医・法医）. **Specific Activities of ABO Gene Mediated Glycosyltransferases and Blood Group Substances:** S. MIYAZAKI, T. NAKAJIMA, T. KOGURE and K. FURUKAWA (Dept. Legal Med., Sch. Med., Gunma Univ., Maebashi)

Watkins らは，A 型および B 型ヒト血清中の型合成酵素に *in vitro* で弱い B 型および A 型合成活性があることを認めている．そこで生体内での作用を検討した．ヒトの型物質や唾液について凝集阻止試験および吸収試験を行ったところ，とくに胃粘膜において A 型に弱い B 型活性，B 型に弱い A 型活性が認められ，また O 型胃粘膜のなかにも A, B 型活性が認められるものがあった．これらの型活性は，*Cl. tertium* A の A 分解酵素および *Cl. sporogenes* Maebashi の B 分解酵素の作用で消失し新たに H 活性が出現ないし増強することからも，A 型および B 型活性であることを確認した．したが

って生体内ではとくに胃粘膜において A および B 遺伝子支配の合成酵素の持つ reciprocal な B および A 合成活性に基づく微量の産生物が存在する可能性が示唆された．さらに O 型の持つ O 遺伝子の直接の産物に弱い A や B 合成活性が存在し，胃粘膜では作用が現れている可能性が考えられた．そこで血清中の合成酵素活性を O 型パパイン処理血球を基質血球として型転換でみると，B 型血清による A 型活性血球への転換などに加え O 型血清による A 型活性血球への転換が認められた．

I-3. Genetic Analysis of Human Red Cell Proteins by Two-Dimensional Gel Electrophoresis: Ikuko KONDO and Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Numerous electrophoretic variants in human red cell proteins have been detected by conventional electrophoresis. To detect these variants effectively by one operation and to find new polymorphic polypeptides, human red cell proteins from 100 individuals were analyzed by two-dimensional gel electrophoresis (two-DE) systems (Hamaguchi *et al.*, 1981; Kondo *et al.*, 1984). About 100 polypeptide spots were detected in two-DE pattern. Polymorphisms were observed in four polypeptides with molecular weight (mol. wt.) of 38,000, 33,000, 25,000, and 20,000. The data on the fractionation of red cell proteins by Sephadex G-100 column suggest that all the four polypeptides are present as homodimers in cells. Family studies indicate that phenotype of each polypeptide is determined by two common alleles at an autosomal locus. The polypeptide with mol. wt. of 33,000 was identified as a subunit of esterase D which was also detected previously in 2-DE pattern of lymphocyte proteins (Kondo *et al.*, 1984). The polypeptide with mol. wt. of 38,000 was the same as the polymorphic polypeptide which was detected previously in lymphocyte proteins (Kondo *et al.*, 1983). The polypeptides with mol. wt. of 25,000 and 20,000 are new polymorphic polypeptides, because they are different from acid phosphatase and glyoxalase 1 in phenotypes in same individuals and because they are different from gluthathione peroxidase in the subunit number. The polypeptides with mol. wt. of 25,000 and 20,000 are designated temporarily as red cell cytosol 25K polypeptide (RCP_{25}) and cytosol 20K polypeptide (CP_{20}), respectively, since the RCP_{25} is present in only red blood cells and since CP_{20} exists in cytosol of various cells including lymphocytes and fibroblasts. The gene frequencies of two common alleles for RCP_{25} were estimated to be 0.780 and 0.220, respectively, and those for CP_{20} 0.955 and 0.045, respectively, in a Japanese population.

I-4. Study of Genetic Variation among the Japanse (in Hiroshima, Nagasaki) Using Two-Dimensional Electrophoresis. III. Proteins in Erythrocyte Lysates: Norio TAKAHASHI, Jun-ichi ASAKAWA, Mikio FUJITA, Yuko NAGAHARA, Yoshiko TANAKA and Chiyoko SATOH (RERF, Hiroshima)

We have been carrying out studies to evaluate the effects of A-bomb radiation on gene mutation of germ cells as reflected in mutations at the protein level.[1] Recently a pilot

study was begun using two-dimensional electrophoresis (2D-PAGE) to examine as many loci as possible from each subject. Genetic variation of proteins in erythrocyte lysates were studied. Erythrocyte samples were obtained from participants in the RERF Biochemical Genetics Study (Hiroshima: 50, Nagasaki: 50 unrelated individuals.) To confirm the inheritance of variants, electrophoretic study was made of mother-father-child trios. The ISO-DALT system of Anderson *et al.* was employed for the electrophoresis, and the gels were stained according to the modified method of Wray *et al.*[2] Forty unidentified peptides were scored from the large number of visible peptides, and six kinds of genetic variants were observed, occurring at a frequency of over 5%. This report describes four kinds of variants having molecular weights less than 35K dalton. Three of them (C20, D14 and D17; according to our tentative designation) seem to be identical with the variants in a predominantly Caucasoid population reported by Rosenblum *et al.*[3] The frequencies of two variants (C20 and D14) in the Japanese are significantly lower than those reported by Rosenblum *et al.*[3] Even though the numbers examined are still relatively small, ethnic differences appear to be emerging.

1) Neel, J.V. *et al.* 1980. *Proc. Natl. Acad. Sci. U.S.A.* **77**: 4221.
2) Wray, W. *et al.* 1981. *Anal. Biochem.* **118**: 197.
3) Rosenblum, B.B. *et al.* 1984. *Am. J. Hum. Genet.* **36**: 601.

I-5. Study of Genetic Variation among the Japanese (in Hiroshima, Nagasaki) Using Two-Dimensional Electrophoresis. IV. Platelet Proteins: Jun-ichi ASAKAWA, Norio TAKAHASHI, Mikio FUJITA, Satomichi KANEOKA, Eiko NISHIKORI and Chiyoko SATOH (RERF, Hiroshima)

We have studied genetic variation of platelet proteins using O'Farrell's two-dimensional electrophoresis method to obtain as much genetic information as possible from each individual. Platelets, isolated from peripheral blood of 50 subjects and their parents residing in Hiroshima, were solubilized in a solution containing 9 M urea, centrifuged and the supernatant was used for electrophoresis. In order to confirm whether the variants detected were inherited or not, electrophoresis was performed for samples from mother-father-child trios using the ISO-DALT system developed by Anderson *et al.* The modified silver staining method of Wray *et al.*[1] was used to stain the gels. Among a large number of peptides selected at random on each gel, 63 types of peptides having high reproducibility were examined for the variation. Variants were detected among 11 types of peptides, and all hereditary in nature. Of these, six variants had gene frequencies between 0.43 and 0.13. There are many peptides which appear to be identical in two-dimensional electrophoretograms of platelets and lymphocytes. Three types of variants among those detected in this study, from their relative location and gene frequency, appear to be identical to variants detected in lymphocytes by Hamaguchi *et al.*[2] and Kondo *et al.*[3]

1) Wray, W. *et al.* 1981. *Anal. Biochem.* **118**: 197.
2) Hamaguchi, H. *et al.* 1981. *Hum. Genet.* **59**: 215.
3) Kondo, I. *et al.* 1984. *Hum. Genet.* **66**: 244.

I-6. A Mutant of Triosephosphate Isomerase (TPI) with Reduced Activity: Chiyoko SATOH, Jun-ichi ASAKAWA, Mikio FUJITA, Ryuji HAZAWA, Akiko MIURA, Hideo OMINE and Naomi MASUNARI (RERF, Hiroshima and Nagasaki)

To evaluate the potential genetic effects of the atomic bombs, a search has been underway for mutations resulting in marked loss of activity of 11 erythrocyte enzymes in 3,454 children born to A-bomb survivors and 4,209 control children. Hemolysates were prepared following the ICSH method and enzyme activity was determined as previously described.[1] During this process, 1,906 children of exposed parents and 2,401 control children were examined for TPI activity. Mean activity $\pm$ SD was 2,215 $\pm$ 193 (IU/gHg) for the former group and 2,210 $\pm$ 186 (IU/gHb) for the latter. Five variants whose activities $\leqslant$ 66% of normal were encountered in the former and 3 in the latter. Their activities were 37%, 48%, 50%, 53% and 65%, and 43%, 52% and 54% of the respective means. A parent exhibited a similar variant in seven of the cases but not for Case 5 of the exposed group. Activities of mother and father of Case 5, a female Nagasaki child, were 92% and 100%, respectively. Electrophoretic pattern and thermostability of all three were normal. As no parental exclusion was apparent judging from blood, protein and HLA typings, the variant of the child is considered to have originated from mutation in the germ cell of the father or mother. The father's estimated air dose from the bomb was 17 rad (γ-ray) while the mother was not exposed to. From an accumulated total of 44,010 equivalent locus tests for the genes encoding for the 11 enzymes, with the detection of 1 mutant among the children of exposed parents, the mutation rate is 2.3×10^{-5} per locus per generation. For the controls, there were no mutations observed in 47,961 equivalent locus tests. Mutation rates previously obtained in our electrophoretic screening program[2] were 0.52×10^{-5} for children of exposed parents and 0.66×10^{-5} for the controls, considerably less than that obtained in screening for enzyme activity.

1) Satoh *et al.* 1983. *Am. J. Hum. Genet.* **35**, 656.
2) Satoh *et al.*, Proc. 25th Late A-bomb Effects Research Meeting. June 3rd, 1984.

I-7. Genetico-Biochemical Studies of Human Erythrocyte AMP Deaminase: I. NISHIGAKI,[1,2] T. ITOH,[3] N. OGASAWARA,[3] M. BAMBA[2] and T. TAKINO[1] ([1]Dept. Intern. Med., Kyoto Pref. Univ. Med., Kyoto; [2]Kohoku-Sogo Hosp., Kyoto; [3]Dept. Genet. Biochem., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

A new muscular disease, which might be attributable to the lack of AMP deaminase in muscle, was reported in patients with muscular weakness and cramping after exercise by

Fishbein *et al.* (1978). No hereditary description of AMP deaminase in erythrocytes however, has been so far published. In the present study we report the genetico-biochemical characteristics of erythrocyte AMP deaminase together with a first description of the complete deficiency of erythrocyte AMP deaminase. AMP deaminase was assayed colorimetrically in 530 blood samples by estimating production of ammonia with phenol/hypochlorite reagents. The mean value of activity was calculated to be 13.08 ± 3.14 units/gHb. Of great interest was the finding of individuals having about one half of the activity of control in a frequency of about 1/30. The value in these individuals averaged 5.97 ± 1.08. One of the parents of these individuals also showed lower AMP deaminase activity as their children but the other had activities within the normal limits. We further assayed AMP deaminase activity of 5,500 blood samples by a semi-quantitative method and found a proband with complete deficiency of this enzyme. He was quite healthy, with no evidence of hemolysis. The data on the family of this proband provided evidence for autosomal recessive inheritance of erythrocyte AMP deaminase deficiency. The frequency of mutant gene is approximately 0.017, resulting in one complete deficiency in 3,000–3,500 population. Thus the erythrocyte AMP deaminase deficiency must be one of the most common enzyme deficiences, designated as non-disease by Beutler.

I-8. A Japanese Family with Hereditary Methemoglobinemia due to NADH-Cytochrome b_5 Reductase Deficiency Restricted to Blood Cells: Kiyoh TANISHIMA (Dept. Med. Technol., Paramed. Sch., Kanazawa Univ., Kanazawa), **Kazuhiro MAWATARI, Yoshimasa YONEYAMA** (Dept. Biochem., Kanazawa Univ., Kanazawa) **and Hitoshi OHKUWA** (Dept. Intern. Med., Kurobe City Hosp., Kurobe)

A family with hereditary methemoglobinemia due to NADH-cytochrome b_5 reductase deficiency restricted to blood cells was found and studied. The patients are two fraternal males at age 18 and 20. They had slight cyanosis of lips, nail beds and cheeks, and erythrocytosis. No cardiovascular, pulmonary and neurological abnormalities, however, were observed. Methemoglobin contents of their blood specimens were 24.0% and 25.3–26.7%, respectively. The activities of diaphorase, ferrihemoglobin reductase and cytochrome b_5 in erythrocytes, leucocytes and platelets from the patients, and their paternal and maternal family members were measured according to the methods of Scott, Hegesh and Haltquist, respectively. The activities of the enzymes in these cells from two patients were found to be essentially absent. The activities of the enzymes from their parents and some of their paternal and maternal family members were intermediate between those of the patients and those of the normal control. These family members seem to be heterozygous. Isoelectric focusing study failed to detect no enzyme activities in erythrocyte

hemolysates derived from the patients, while the heterozygotes showed normal patterns. Such a family with a cytochrome b_5 reductase deficiency not restricted to erythrocytes but without neurological involvement has not been observed in Japan. The present cases seem to have a different form of hereditary methemoglobinemia from either classical form with erythrocyte type reductase deficiency and generalized type reductase deficiency with associated neurological involvement.

I-9. アカタラセミアマウスのカタラーゼのゲル沪過および等電点電気泳動による分析： **佐藤征紀・田中由紀子・緒方正名**（岡山大・公衆衛生）． **Property of Acatalasemic Mouse Catalase by Gell Filtration and Isoelectric Electrophoresis:** Yukinori SATO, Yukiko TANAKA and Masana OGATA (Dept. Public Health, Okayama Univ., Okayama)

酵素活性が正常の約2.4%ときわめて低値であるアカタラセミアマウスの赤血球カタラーゼ蛋白分子は，正常とは構造上の変異があるとされているが，今回，薄層ゲル沪過クロマトグラフィーで分画したうえ，等電点電気泳動法で分析を行った．正常，アカタラセミア同型接合体，アカタラセミア異型接合体の溶血液をゲル沪過法で展開し，H_2O_2 で発泡させた状態ではアカタラセミア同型接合体よりも正常のほうが活性も強く分子量も大きいと推察された．また，アカタラセミア異型接合体はその両親のほぼ中間を示した．次に，展開された各カタラーゼ酵素を等電点電気泳動法で分離し KI 染色を行うと，アカタラセミア同型接合体の赤血球カタラーゼの等電点はアルカリ側に近く，正常は酸性側にバンドが形成され，アカタラセミア異型接合体はほぼその中間を示した．この傾向は，溶血液からエタノール・クロロホルムで分離し凍結乾燥した赤血球カタラーゼを泳動した結果でも同様に見られた．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と結論できる．

I-10. 東京地区における G6PD 異常症の頻度調査： **高橋圭介・藤井寿一・三輪史朗**（東大・医科研・病態薬理）． **Frequency of G6PD Deficiency in Tokyo Area:** Keisuke TAKAHASHI, Hisaichi FUJII, and Shiro MIWA (Dept. Pathol. Pharmacol., Inst. Med. Sci., Univ. Tokyo, Tokyo)

〔緒言〕 Glucose-6-phosphate dehydrogenase (G6PD) 異常症は，世界で最も頻度が高い赤血球酵素異常症である．無症状のものも多いが，薬剤惹起性急性溶血発作を起こすことで知られる．人種間や地域によって頻度が異なり，さらに異なった変異酵素が存在する．ゆえに G6PD 異常症のスクリーニングは血液学的，人類遺伝学的に重要である．今回，東京地区においてスクリーニングを行い，3 例の異常症を発見したので報告する．〔方法〕 スクリーニング法としては，ホルマザンリング法とデンプンゲル電気泳動法を用いた．前者はわれわれが新しく開発した方法で，反応液を加えた寒天上に沪紙にしみこませた検体を置き，青色ホルマザンのリングの大きさを測定する方法である（*Acta Haematol. Jpn.* 47: 185–188, 1984）．〔結果・考察〕 日赤医療センターの外来受診者および入院患者の男性のみを対象として 4,824 名の検索を行い，3 例の G6PD 異常症を発見し，頻度は 0.06% であった．すでにわれわれが行った山口県下での頻度 0.1～0.5% に比べ低い結果となった．WHO の方法に従って酵素学的検索を行ったところ，3 例とも酵素活性は正常の約 50% で，電気泳動易動度

が速く，われわれが山口地方で発見，報告した G6PD Konan と同一であった．この変異酵素は，日本人に比較的広く分布すると考えられる．日本人の起源を考えるうえで重要な所見と思われ，今後，変異酵素の検索を続け gene-flow を検討する予定である．

I-11. Abnormal Hemoglobins in Kobe District: Kazuo HIDAKA, Iwao IUCHI, Shunichi SHIMASAKI (Kawasaki Med. Sch., Kurashiki) **and Wataru MIZUTA** (Kobe Muni. Cent. Hosp., Kobe)

A survey of hemoglobinopathy in Kobe district was conducted in the individuals totaling 44,500 by isoelectric focusing for the past four years. Fifteen abnormal hemoglobins were discovered from fifteen families. Ten variants of them were a α chain anomaly, five of them β chain anomaly. Primaly structure of these abnormal hemoglobins was established as follows: 1) three of Hb Ube-2 (α68 Asn→Asp), 2) one of each Hb Syracuse (β143 His→Pro), Hb Nunobiki (α141 Arg→Cys), Hb Albany-Suma (α11 Lys→Asn), Hb Coushatta (β22 Glu→Ala), Hb Handa (α90 Lys→Met), Hb Ankara (α10 Ala→Asp), Hb J Habana (α71 Ala→Glu), Hb Riyadh (β120 Lys→Asn) and Hb L Ferrara (α47 Asp→Gly). The variant demonstrated the characteristic nature as follows; 1) A proband (22-year-old female) with Hb Syracuse revealed polycythemia without cyanosis (RBC $6.29\times10^{12}/l$, Hb 15.4 g/dl, PCV 0.52 l/l) due to its high oxygen affinity. Log P_{50}, Hill's constant n and Bohr effect in oxygen equilibrium curve (OEC) were -0.01 (Hb A$=1.02$), 1.25 (Hb A$=2.54$) and -0.33 (Hb A$=-0.44$), respectively. Hb Syracuse was separable only by isoelectric focusing and DE-52 column chromatography. 2) The carrier (41-year-old male) with Hb Nunobiki revealed a tendency toward polycythemia (RBC $5.16\times10^{12}/l$, Hb 16.1 g/dl, PCV 0.46 l/l) since the variant has a high oxygen affinity; log P_{50} 0.360 (1.08), Hill's n 1.25 (2.88) and Bohr effect -0.34 (-0.45). Hb Albany-Suma showed slightly but significantly high oxygen affinity; log P_{50} 0.893 (0.980), Hill's n 1.60 (2.40) and Bohr effect -0.47 (-0.51). Isoelectric focusing gave a single one fast-moving band in fresh blood while old specimen (stored at 4°C for more than one month) gave two bands for the sake of partial oxidation of a newly introduced C-terminal amino acid in α chain. Hb Ube-2 is the variant distributed sporadically among Japanese. The detection rate of abnormal Hbs in this district was one per 2,960 individuals with a frequency of 0.03%. This value seems the same as other districts in Japan.

I-12. Neonatal Mass-Screening of Hemoglobin Variants Using Dried Blood on Filter Paper: Akira HAYASHI and Yoshinao WADA (Osaka Med. Cent. Res. Inst., M.C.H., Osaka)

Using the dried blood on filter paper which was collected for the neonatal mass-screening of inborn errors of metabolism, the mass-screening of hemoglobin variants was performed

in Osaka Prefecture, Japan. The structural analysis of the detected hemoglobin variants was carried out with mass spectrometry using the same dried blood. (1) Mass-screening of hemoglobin variants: the globin chains were extracted from a 3 mm disc of the dried blood spot in the solution containing 8 M urea–10% (v/v) 2-mercaptoethanol, placed on the polyacrylamide slab gel containing 8 M urea–3% v/v) carrier ampholite, and isoelectric focusing was performed. (2) Structural analysis of the abnormal globin chain: the abnormal globin chain was isolated on CM-cellulose column, digested with trypsin, and the peptide mixture obtained was analyzed by secondary ion mass spectrometry. All the newborns in Osaka Prefecture are under examination. So far, 11 α variants and 38 γ variants were detected out of 50,000 newborns, nearly at the rate of 1 out of 1,000. Fifteen of the samples detected were analyzed and the primary structures were determined. One sample of Hb F Izumi (Kotobuki), $^{A}\gamma^{I}$ 6 Glu→Gly and 2 samples of Hb F Fuchu, $^{G}\gamma^{I}$ 21 Glu→Gln were determined as new variants. All the others were identified as Hb Yamaguchi, $^{A}\gamma^{T}$ 80 Asp→Asn, which was proved to be a genetic polymorphism widely distributed among the Japanese.

I-13. ヒト γ グロビン遺伝子座数の多様性：島崎俊一・井内岩夫・日高和夫（川崎医大・生化）. **Diversity of Human γ Globin Gene Loci Including Quadruplicated Arrangement:** S. SHIMASAKI, I. IUCHI, K. HIDAKA (Dept. Biochem., Kawasaki Med. Sch., Kurashiki)

ヒト γ グロビン遺伝子は，$^{G}\gamma$ と $^{A}\gamma$ の二重連鎖構造を形成し，両遺伝子間の DNA 塩基配列は実質的に相同であるため細胞分裂時に遺伝子不等交叉を惹起し，γ グロビン遺伝子座数が変化することがある．われわれはこのことを調べるため，健康成人 104 名（日本人 103 名，中国人 1 名）の白血球 DNA を用いて，ブロットハイブリダイゼーション法でそれぞれの γ グロビン遺伝子座数を決定した．すなわち，全例中，5 例の三重連鎖ヘテロ接合体，2 例の単遺伝子座ヘテロ接合体を検出し，さらに，たまたま検査に含まれた 1 名の中国人は四重連鎖ヘテロ接合体であることが判明した．四重連鎖構造は，世界で最初の例であった．三重連鎖構造の検出頻度が，同じ確率で伝播が期待される単遺伝子座構造のそれより高いのは，胎児期において単遺伝子座構造が γ サラセミア等の淘汰圧を受けていることによるのかもしれない．これらの変異型遺伝子連鎖構造は，制限酵素地図の成績をもとにし，DNA 塩基配列の 5′ 側より，三重連鎖が $-^{G}\gamma-^{G}\gamma-^{A}\gamma-$，四重連鎖が $-^{G}\gamma-^{G}\gamma-^{G}\gamma-^{A}\gamma-$，単遺伝子が $-^{A}\gamma-$ であることが判明した．このことから今回検出したすべての変異連鎖構造は，第三エクソン内に存在するアミノ酸配列番号 136 番のトリプレットコドンより 5′ 側で遺伝子不等交叉を起こしていると考えられた．

I-14. Direct Comparison of $^{A}\gamma^{T}$ Gene Frequencies between the Japanese and the Ainu as Assessed from Polymorphic Trait of Fetal Hemoglobin in the Adult Blood: **Yuriko NOZAWA, Tadashi SENO, Mikio MATSUI, Takayoshi TSUCHIYA, Akihiko KAJITA** (Dept. Biochem., Dokkyo Univ. Sch. Med., Tochigi) **and Keiichi OMOTO** (Dept. Anthropol., Univ. Tokyo, Tokyo)

The introduction of high pressure liquid chromatography (HPLC) for the separation of three types of γ chains in the human fetal hemoglobin (HbF), *i.e.* the $^{G}\gamma$ (75 Ile, 136 Gly), the $^{A}\gamma^{I}$ (75 Ile, 136 Ala) and $^{A}\gamma^{T}$ (75 Ile, 136 Ala) and $^{A}\gamma^{T}$ (75 Thr, 136 Ala) chains, has greatly facilitated analysis of the γ chain heterogeneity in the cord blood samples. Using this HPLC methods, we reported recently that the $^{A}\gamma^{T}$ gene frequency of Japanese newborn baby born in Tochigi area is 0.165, which locates between those of Caucasoid (0.224) and of Negroid (0.102). In the meantime, we succeeded in obtaining the frequency in adult blood in which content of the HbF is known to be less than 1% of total hemoglobin using the alkali denaturation procedure. The frequency obtained was 0.158 in good agreement with that of the newborn. It has been known that the Ainu population possesses a genetic composition basically similar to that of the Japanese, despite the marked difference in morphological characteristics. To study on the process of genetic differentiation of human populations, we compared the frequencies of $^{A}\gamma^{T}$ gene between both races. Hemolysate was prepared from frozen erythrocyte of the adult Ainu which had been stored under −20°C for 10–15 years. Bulk of HbF isolated by the procedure described above was subjected to HPLC. From the elution pattern three types of the chains were quantitated. Result from 129 adult Ainu revealed that about 48% of the Ainu carry the $^{A}\gamma^{T}$ chain. The gene frequency of the $^{A}\gamma^{T}$ was found to be 0.279, which is close to that of the Caucasoid and higher than that of the Japanese (0.158–0.178).

I-15. A Single Family Having Both Atypical Hp and ABO Blood Types: **Kaoru MORITA** (Dept. Legal Med., Sch. Med., Toho Univ., Tokyo)

A family group, whose proband (76 years old male) was hard to determine his ABO blood groups, was recently referred to our division. Our checking of his blood and saliva specimens showed that he belongs to group Bm. One of his two children was found to belong to groug A_1Bm. During the processes of our examination of the entire family, it was discovered that the haptoglobin (Hp) type of the mother was 2-2, whereas the type of both two children were 1-1. Real parent-children relationships were confirmed by the examinations of other genetic markers using blood, serum and saliva. We have, therefore, assumed the existence of a silent Hp allele in this family. From that assumption, Hp types of the mother and two children were considered to be 2-0 and 1-0, respectively. Existence of two rare genetic factors in a single family may be a very rare case.

I-16. Acid phosphatase 1 (ACP1) の遺伝子座位の検討：脇田宣治・楢原幸二・高橋幸雄・吉川清志・木村俊介・小田 慈・木本 浩(岡山大・小児). **Regional Mapping of Acid Phosphatase 1 (ACP1) to 2p25.1:** Y. WAKITA, K. NARAHARA, Y. TAKAHASHI, K. KIKKAWA, S. KIMURA, M. ODA and H. KIMOTO (Dept. Pediatr., Okayama Univ., Okayama)

ACP1 遺伝子座位の局在に関しては，現在なお論争されており，2p25 あるいは 2p23 のいずれかに存在すると考えられている．そこで 2p 部分トリソミーの症例で，ACP1 の遺伝子量効果を検討した．症例の臨床症状は，精神運動発達遅延を除き，従来より報告されている 2p 部分トリソミー症候群に類似していた．症例の高精度分染法による核型は，46, XX, dir dup (2)(pter → p25.3: p25.3 → p25.1: p25.3 → qter) で，2p25.1 → 2p25.3 の分節の染色体内重複が認められた．赤血球 ACP の活性測定には，*p*-nitrophenyl phosphate を基質とする Hopkinson らの方法 (1964) を，赤血球 ACP isozyme の同定には，Harris and Hopkinson (1976) の澱粉ゲル電気泳動法を応用した．正常対照 204 例の分析の結果，各 isozyme pattern の活性値は，type A で 131.0±19.4 U/gHb (N=13), type B で 186.7±19.4 U/gHb (N=117), type BA で 157.3±15.9 U/gHb (N=67), type CB で 215.8±19.3 U/gHb (N=5), type CA で 199.7±3.3 U/gHb (N=2) であった．一方，症例は type B で，活性値は 259.1 U/gHb と対照の 1.39 倍に上昇していた．父親は type BA で，活性値は 147.1 U/gHb，母親は type B で活性値は 176.8 U/gHb であり，症例の ACP1 に 1.5 倍の遺伝子量効果が考えられた．なお，赤血球 MDH1 の活性値は正常であった．本症例の検討結果および Human Gene Mapping 7 の報告により，ACP1 の遺伝子座位は 2p25.1 に存在することが強く示唆された．

I-17. Low Voltage Isoelectric Focusing for Phenotyping of Esterase D: Isao YUASA (Dept. Legal Med., Tottori Univ. Sch. Med., Yonago), **Nobuto TAMAKI** (Sci. Crime Lab., Tottori Pref. Police Hq., Tottori), **Kazuyuki SUENAGA** (Yamaguchi Red Cross Hosp., Yamaguchi) **and Keiichi ITO** (Yamaguchi Red Cross Blood Cent., Yamaguchi)

In this study a new isoelectric focusing method is presented for the discrimination of the three common phenotypes of esterase D (ESD) as well as confirmation of ESD 7. When isoelectric focusing was done at 4 W, 1,000 V and unlimited mA for 70–80 min on a gel (110×200×0.5 mm) containing 2.4% Ampholine, pH 4–6.5, the clear and unambiguous differences among the three common ESD phenotypes were demonstrated. Two homozygotes, ESD 1 and ESD 2 consisted of a major cathodal band and a minor anodal band, whereas heterozygote, ESD 2-1 was represented by five prominent bands including heteromeric bands. The clearly distinct patterns of the heterozygous phenotypes ESD 7-1 and ESD 7-2 were easily recognizable. Using this method the distribution of ESD phenotypes and allele frequencies in 504 unrelated Japanese living in Yamaguchi prefecture, Western Japan was investigated. A new rare variant was identified which was apparently identical with ESD 2-1 in agarose gel electrophoresis. After IEF the variant was slightly cathodal to ESD 2. The obtained gene frequencies were $ESD^*1=0.600$, $ESD^*2=0.389$ and ESD^*7

=0.011. The evidence for geographical cline for ESD allele frequency in Western Japan was presented. *ESD*2* frequency proved to increase gradually from east (0.342 in Tokyo) to west (0.389 in Yamaguchi).

I-18. 13q 部分トリソミーにおける esterase D (ESD) の遺伝子量効果：高橋幸雄・楢原幸二・脇田宜治・吉川清志・木村俊介・木本　浩（岡山大・小児），**笠井良造**（旭川児童院）. **Gene Dosage Effects of Esterase D (ESD) in Partial Trisomy 13q:** Y. TAKAHASHI, K. NARAHARA, Y. WAKITA, K. KIKKAWA, S. KIMURA, H. KIMOTO (Dept. Pediatr., Okayama Univ., Okayama) and R. KASAI (Asahigawa Jidoin Child. Hosp., Okayama)

ESD および網膜芽細胞腫の遺伝子座位は，13q14.1 に決定されているが，subband での局在は不明である．高精度分染法を応用した染色体分析で，13q14.11→qter のトリソミーと同定された 3 症例について，ESD の遺伝子量効果を検討した．赤血球 ESD 活性の測定には Sparkes らの方法 (1979) を，ESD isozyme の分析には澱粉ゲル電気泳動 (Harris と Hopkinson, 1976) とを応用した．正常対照 158 例における ESD 活性値は，type 1 で 4.27±0.51 U/gHb (N=63), type 2-1 で 3.32±0.39 U/gHb (N=77), type 2 で 2.20±0.26 U/gHb (N=18) であった．一方，3 症例の ESD isozyme pattern と活性値は，症例 1 では type 1, 7.39 U/gHb, 症例 2 では type 2-1, 4.86 U/gHb, 症例 3 では type 2-1, 5.74 U/gHb であった．ESD 活性値および isozyme の泳動パターンより，3 症例の ESD 遺伝型は，症例 1 では 1-1-1, 症例 2 では 2-2-1, 症例 3 では 2-1-1 であると考えられた．Ward ら (1984) は，ESD および網膜芽細胞腫の遺伝子座位を 13q14.11 と報告しているが，本症例では，切断点が 13q14.11 の近位部に認められたので，ESD の遺伝子座位は 13q14.11 の遠位側 2/3 に存在すると推測される．また，ESD isozyme の泳動パターンの解析から，ESD の遺伝子量効果の推測が可能であることを報告した．

I-19. 網膜芽細胞腫患者 50 例における赤血球エステラーゼ D 活性の検索：藤木慶子・桑原洋子・中島　章（順天堂大・眼科），**西垣逸郎**（京都府医大・内科），**池内達郎**（東医歯大・難研）. **Esterase D Activity in the Erythrocytes from 50 Patients with Retinoblastoma:** K. FUJIKI, Y. KUWABARA, A. NAKAJIMA (Dept. Ophthal., Juntendo Univ., Sch. Med., Tokyo), I. NISHIGAKI (Dept. Med., Kyoto Pref. Univ. Med., Kyoto) and T. IKEUCHI (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

網膜芽細胞腫 (Rtb) 患者 50 例とその両親および同胞 102 名の赤血球エステラーゼ D (ESD) を定性的，定量的に測定し，合わせて染色体の分析を行った．結果は 50 例中 1 例のみに No. 13 染色体の欠失を認め，欠失部位は q12.3→q21.2 で q14 を完全に含み，患児の ESD 活性値は 2-1 型の 1.4 unit/gHb であり，2 型の平均値の約 1/2 であることから，母親由来の染色体の欠失であることが確認された．残る 49 例については型別の ESD 活性値に異常は認められず，全員正常範囲であった．また，染色体分析の済んだ患者について欠失などの異常は認められなかった．以上の結果は，Rtb 患者における 13q の欠失は少なく，従来の知見と矛盾しないが，ESD 活性値を測定することによって，染色体分析では見いだせない minor deletion を発見するという試みは果たせなかった．

I-20. A Case Report of the Patient with Retinoblastoma and Chromosome 13q Deletion: Assignment of the Gene for LCP_{64} on Chromosome 13: **Ikuko KONDO,[1] Kenji SHIN,[2] Sachiko HONMURA,[3] Hachiro NAKAJIMA,[4] Hitoshi TAKITA[2] and Hideo HAMAGUCHI[1]** ([1]Dept. Hum. Genet., [2]Dept. Pediatr., [3]Dept. Ophthal., Univ. Tsukuba, Ibaraki; [4]Dept. Foren. Med., Tokyo Med. Dent. Univ., Tokyo)

Five percent of the patients with retinoblastoma (Rb) have a deletion involving the region of the chromosome 13q14.1 (Vogel, 1979) and the locus for Rb gene (Rb-1) is closely linked to the locus for esterase D (EsD, EC 3.1.1.1) assigned to the chromosome 13q14.1 (Sparkes *et al.*, 1983). Here, we report a case who was predicted to have Rb from the genetic analyses of chromosome and EsD phenotype. A three month-old girl was admitted to the Tsukuba University hospital for the evaluation of developmental retardation and cyanosis. At three month of age, her weight was 2,200 g and she had many unusual phenotypes including doligocephaly, epicanthus, ptosis, depressed nasal bridge, micrognathia, short webeed neck, short fifth fingers with clinodactyly and single crease, and simian lines in both hands. A cardiac murmur was heard and she was diagnosed to have the tetralogy Fallot by cardiac examinations. The karyotype of the patient was 46,XX,der(13)(q14.1-q32.1), though both the parents had normal karyotypes. As expected, the phenotype of EsD derived from one of the parents, the father in this case, was not detected in red blood cells by two-dimensional gel electrophoresis (2-DE), indicating that the EsD gene from the father was deleted in the abnormal chromosome 13. In addition, lymphocyte cytosol 64K polypeptide (LCP_{64}) (LCP 1; McKusick catalogue No. 15343) that is a new polymorphic polypeptide detected by 2-DE (Hamaguchi *et al.*, 1982) was also deleted in the lymphocyte proteins from the patient. The probability of paternity was over 0.999 calculated based on 22 genetic markers. These data indicate that the gene for LCP_{64} is located in the region q14.1-q32 of chromosome 13.

I-21. 13q− 症候群患児に発症した網膜芽細胞腫の染色体とエステラーゼ Dについて： **池内達郎**（東医歯大・難研・細胞遺伝），**近藤郁子・浜口秀夫**（筑波大・基礎医・人類遺伝）． **Chromosomes and Esterase D Phenotypes in Retinoblastoma Tumor Cells Derived from a Patient with 13q− Syndrome:** T. IKEUCHI (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo), I. KONDO and H. HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

発癌の多段階説に従えば，初発突然変異を先天的に保有する個体に発症すると考えられる遺伝性腫瘍は，細胞の癌化機構を解明する上で貴重な試料である．No. 13 染色体に連鎖する esterase D (ESD) や DNA 多型 (RFLPs) を指標として用いた最近の網膜芽細胞腫 (RB) の研究は，初発突然変異（主遺伝子）のヘテロ接合型からホモ接合型またはヘミ接合型への転換が，細胞の癌化につながることを示唆している．しかし，No. 13 染色体上の可視的な欠失を伴う患者に発症した腫瘍について検討し

た報告はまだない．本症例（3 カ月女児）は，両側性 RB のほかに，13q− 症候群に合致した種々の先天奇型を有する（近藤ら，本学会報告）．臭化エチジウム前処理法を用いた染色体の高精度バンド解析（550～850 バンド期）により，欠失領域は 13q14.1→q32 と判定された．両親の核型は正常．二次元電気泳動法により検出される ESD の表現型は，母 1-2 型，父 1-1 型，患児 2 型であった．すなわち，患児では父親由来の ESD 座位が欠損しており，このことは Q-染色法による異型性 No. 13 染色体の観察からも立証された．患児の右眼球摘出により得た腫瘍の染色体を短期培養により検査したところ，全細胞に 46,XX, 13q−, 1p+ の核型を得た．摘出時の腫瘍の一部分について ESD の表現型を調べたところ，正常細胞と同様 2 型であった．すなわち，本試料では，腫瘍細胞における欠失型突然変異のホモ接合化またはヘミ接合化の確証は得られなかった．

I-22. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. VIII. Evidence for the Genetic Linkage of the Locus for LCP_{64} with the EsD Locus: Hideo HAMAGUCHI and Ikuko KONDO (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Lymphocyte cytosol polypeptide with molecular weight of 64,000 (LCP_{64}) (LCP 1: McKusick catalogue No. 15343) is a human polymorphic polypeptide which can be detected in peripheral blood lymphocytes by two-dimensional gel electrophoresis (two-DE) (Hamaguchi *et al.*, 1982). The phenotype of LCP_{64} is determined by two common codominant alleles at an autosomal locus. Recently, we were able to assign the gene for LCP_{64} to the chromosomal region 13q14.1-q32 by deletion mapping (Kondo *et al.*, 1985). Therefore, we examined the linkage between LCP_{64} and esterase D (EsD). The genotypes of LCP_{64} and EsD were analyzed by two-DE of peripheral blood lymphocyte proteins. Among 90 families examined, four were informative for the linkage analysis of the loci for LCP_{64} and EsD. There were no proven recombinants. The linkage data were analyzed by the "lod score method of Morton" as described by Meynand-Smith *et al.* The summed lod score is 4.221 at zero recombination. This result indicates that the locus for LCP_{64} is closely linked to the EsD locus and the subband 13q14.1. The finding also suggests that LCP_{64} is the second polymorphic protein closely linked to retinoblastoma.

I-23. Gene Frequencies of *S*-Adenosylhomocysteine Hydrolase in a Japanese Population: Katsunori AKIYAMA, Shigeki NAKAMURA and Kazue ABE (Dept. Legal Med., Tokyo Women's Med. Coll., Tokyo)

Genetic polymorphism of *S*-adenosylhomocysteine hydrolase (SAHH; EC 3.3.1.1, synonymous with AHCY) was investigated in a total of 214 red blood cell samples from unrelated Japanese using starch gel electrophoresis and enzyme-specific staining procedures. Three common phenotypes were observed which corresponded to SAHH 1, SAHH 2-1, and SAHH 2, controlled by two alleles, *SAHH*1* and *SAHH*2*. The estimated gene frequencies of *SAHH*1* and *SAHH*2* in Japanese were 0.953 and 0.047, respectively. There

were no variant types distinct from the three common phenotypes. These results are very similar to the finding in European samples (*SAHH*1*=0.96, *SAHH*2*=0.04) reported by Bissbort *et al.* (1983).

I-24. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. IX. Genetic Polymorphism of the Mitochondrial 68K Polypeptide: **Kimiko YAMAKAWA,[1] Yasuko YAMANOUCHI,[1] Hideo HAMAGUCHI,[1] Ikuko KONDO,[1] V. Darley Usmar,[2] Masanao SHIBASAKI,[3] and Keiji FUJII[4]** ([1]Dept. Hum. Genet., [2]Dept. Biochem., [3]Dept. Pediatr., [4]Dept. Pathol., Univ. Tsukuba, Ibaraki)

We have reported that a cellular polypeptide with mol. wt. of 68,000 has three common phenotypes detected in peripheral blood lymphocytes by 2-D gel electrophoresis (Yamada *et al.*, 1983). In this study, extensive family and population studies on the polypeptide were performed. In addition, cellular localization of the polymorphic 68K polypeptide was analysed. Family and population studies indicated 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 0.63 and 0.37, respectively. The polypeptide was enriched in the mitochondrial fraction, and treatment of lymphoblastoid cells with the K^+ ionophore nonactin resulted in the loss of the polypeptide. Comparing with the other polymorphic mitochondrial proteins, the polypeptide differed from GOT in the mol. wt. and from ME (malic enzyme) in quantitative cell and tissue distributions. These data suggest that the 68K polypeptide is a new polymorphic mitochondrial protein. We propose that the polypeptide is temporarily designated as mitochondrial 68K polypeptide (MTP68) until the elucidation of its physiological role.

I-25. Mitochondrial DNA Polymorphism in Japanese. I. Analysis with Restriction Enzymes of 6 Base Pair Recognition: **Satoshi HORAI, Takashi GOJOBORI and Ei MATSUNAGA** (Dept. Hum. Genet. Evol. Genet., Natl. Inst. Genet., Mishima)

The mitochondrial DNA(mtDNA) from 120 Japanese were analysed with 15 restriction enzymes that recognize six base pairs, of which 11 enzymes showed at least one atypical cleavage pattern. Digestion patterns with HincII and HaeII were highly polymorphic. Seven distinct cleavage patterns were observed for the HincII digestion and five different patterns were found for the HaeII digestion. In addition, EcoRV was found useful to detect polymorphism in our sample, because 5% of individuals showed an atypical cleavage pattern. In digestions with StuI, HindIII, ScaI and XhoI, three different cleavage patterns were observed for each enzyme. While StuI and ScaI were not examined in previous studies, digestion patterns with HindIII, XhoI, SacI, EcoRI and PvuII were reported to be mono-

morphic (Brown, 1980). In the present samples, an atypical cleavage pattern was observed for each of SacI, EcoRI and PvuII. The observed restriction enzyme morphs were classified into 22 types of distinct cleavage patterns. By pairwise comparison of each restriction type, the average number of nucleotide substitutions per nucleotide site (δ) was estimated at 0.00417, which agreed with the values obtained from other human populations in previous studies. There were 11 site gains, of which 7 were transitions and 4 were transversions. Phylogenetic analysis of the present data suggested that the Japanese population conceals a considerably high degree of mtDNA diversity.

I-26. Genetic Variation of Mitochondrial DNA in Japanese: Shinji HARIHARA, Momoki HIRAI and Keiichi OMOTO (Dept. Anthropol., Univ. Tokyo, Tokyo)

The mitochondrial DNA (mtDNA) from 64 Japanese (31 Ainu and 33 Non-Ainu) living in Hokkaido were analyzed for their restriction enzyme fragment patterns. Total DNAs were extracted from blood cells and were digested by five restriction enzymes: AvaII, BamHI, HpaI, HpaII and PvuII. The fragment patterns were then analyzed by Southern hybridization using mtDNA as aprobe. Polymorphisms were detected in mtDNA digested by HpaI and AvaII in the Non-Ainu sample. Three distinct patterns in HpaI digestion corresponded to morph 1, 2 and 4, respectively, reported by Denaro *et al.* (1981). As to AvaII digestion, four distinct patterns were detected, of which three corresponded to morph 1, 2 and 10 reported by Johnson *et al.* (1983). The fourth morph seemed to be a new variant, which appeared to have resulted from the fusion of 3.0 kb and 0.8 kb fragments and the site gain in 9.8 kb fragment generating 6.2 kb and 3.6 kb fragments. In the Ainu population, no variation was thus far detected. Since the present material came from a relatively restricted geographical area, the lack of variation may be due to the bottle neck effect.

I-27. メープルシロップ尿症の2例—その臨床的異質性について—：大和田操・吉田泰祥・津田正彦・西谷　修・北川照男（日大・医・小児）. **Two Cases with Maple Syrup Urine Disease—Clinical Heterogeneity—:** M. OWADA, Y. YOSHIDA, M. TSUDA, O. NISHITANI and T. KITAGAWA (Dept. Pediatr., Nihon Univ. Sch. Med., Tokyo)

メープルシロップ尿症（以下, MSUD）は，現在臨床症状および経過から5型に分類されており，臨床症状の軽重と，本症で遺伝的に障害されている分枝鎖ケト酸脱炭酸酵素（以下，decarboxylase）の残存活性とは並行することが知られている．しかし，個々の症例の症状と残存酵素活性の関連についての詳細な報告は少なく，実際に患者を管理するうえで困難を感ずる場合が少なくない．そこで，われわれは，自験例2例の MSUD における臨床経過を比較するとともに，患者の培養皮膚線維芽細胞を用いて残存する decarboxylase の性質を検討することにより，本症における臨床的ならびに生化学的な異質性について研究し，以下の結論を得た．すなわち，1）診断時に血中分枝鎖アミノ酸の上

昇が著明で，線維芽細胞における decarboxylase の残存活性が正常対照の 6% を示した症例では，軽微な感染で容易に酸血症に陥ったが，同一条件で測定した decarboxylase 活性が対照の 25～35% を示した症例では蛋白認容能が高く，感染時にも急性発作を生じなかった．2) このことは，個々の症例における急性発作の出現し易さについては，残存酵素活性の多寡がある程度の目安になりうることを示している．3) しかし，軽症 MSUD 患者に残存する decarboxylase の K_m は，正常対照の 3 倍と高く，変異酵素である可能性が示唆された．

I-28. Molecular Structure of Argininosuccinate Synthetase Gene: Presence of Highly Repetitive *Alu* Family Sequences in the Introns: Yoshihiro JINNO, Seiji MATUO, Hisayuki NOMIYAMA, Kazunori SHIMADA (Dept. Biochem., Kumamoto Univ. Sch. Med., Kumamoto), **Takeyori SAHEKI** (Dept. Biochem., Sch. Med., Kagoshima Univ., Kagoshima), **and Ichiro MATSUDA** (Dept. Pediatr., Kumamoto Univ. Sch. Med., Kumamoto)

Citrullinemia is a urea cycle disorder resulting from abnormality of argininosuccinate synthetase (AS). It is classified into qualitative and quantitative types. Most of the Japanese patients with citrullinemia belong to the quantitative type, which is characterized by a specific decrease in the amount of AS in liver. To understand the molecular basis of this type of citrullinemia, we initiated study on the structure of AS gene, and isolated numerous phage clones from a human gene library. Restriction enzyme mapping and Southern blotting analysis of three of these clones revealed that they cover a contiguous 30 kilobases DNA including the 5′ end region of AS gene. Sequence analysis of the 5′ end of AS gene revealed presence of a typical TATA box, a consensus sequence for RNA polymerase II. The first exon is 35 bases long and encodes a part of the 5′ noncoding sequence of the AS mRNA. The first intron contains two highly repetitive *Alu* family DNAs; one is situated in the same transcriptional orientation as that of AS gene, and the other in the opposite orientation. Moreover, we found that AS gene is preceded with at least one *Alu* family DNA, and that other introns of AS gene also contain *Alu* family DNAs.

I-29. Analysis of Molecular Basis of Ornithine Transcarbamylase (OTC) Deficiency. (I) Cloning and Structural Analysis of Human OTC Gene: Akira HATA, Teruhisa TSUZUKI, Kazunori SHIMADA (Dept. Biochem., Kumamoto Univ., Kumamoto), **Masaki TAKIGUCHI, Masataka MORI** (Dept. Biochem., Chiba Univ., Chiba), **and Ichiro MATSUDA** (Dept. Pediatr., Kumamoto Univ., Kumamoto)

Ornithine transcarbamylase (OTC) catalyzes the second step of urea cycle and is coded by a gene located on X chromosome. In order to analyze molecular basis of various types of OTC deficiencies, we started to investigate human OTC gene structure.

1) Human placental DNA was analyzed by Southern blot procedure using rat OTC cDNA as a probe. This probe hybridized with the 11, 3.6, 1.3, 1.2, and 0.65 kb fragments present

in the *Eco*RI digests of placental DNA. On an autoradiogram, the density of all these bands was doubly greater in female DNA than in male DNA. This finding suggests that all these fragments are derived from X chromosomes and that there is no sequence homologous to rat OTC cDNA on human autosomes. 2) Using rat OTC cDNA as a probe, ten phage clones were isolated from three different human genomic libraries (one X chromosome library and two independently constructed total genomic libraries). These phage clones covered all the *Eco*RI fragments detected by Southern blotting method. A restriction map of human OTC gene carried by these clones revealed that the size of this gene is at least 60 kb.

I-30. Processing of Human β-Galactosidase in Gm1-Gangliosidosis and Morquio B syndrome: Hiroko KAWASHIMA (Clin. Genet., Kanazawa Med. Univ., Uchinada), **Andre HOOGEVEEN, Alessandra d'AZZO and Hans GALJAARD** (Dept. Cell Biol. Genet., Erasmus Univ., Rotterdam)

The nature of the molecular defect resulting in the β-galactosidase deficiency in different forms of Gm1-gangliosidosis and mucopolysaccharidosis 1V B (Morquio B syndrome) was investigated. Normal and mutant skin fibroblasts were labeled with [^{3}H]leucine and immunoprecipitation studies with human anti-β-galactosidase antiserum were performed, followed by polyacrylamide gel electrophoresis and fluorography. In Morquio B syndrome, the mutation does not interfere with the normal processing and intralysosomal aggregation of β-galactosidase. In cells from infantile and adult, Gml-gangliosidosis is 64K mature lysosomal enzyme with normal catalytic properties but with a reduced ability of the monomeric form to aggregate into high molecular weight multimers. Knowledge of the exact nature of the molecular defect underlying β-galactosidase deficiency in man may lead to a better understanding of the clinical and pathological heterogeneity among patients with different types of Gml-gangliosidosis and Morquio B syndrome.

I-31. Hereditary Plasma Fibronectin Deficiency in 8 Cases in One Family: Akira SHIRAKAMI, Toshio SHIGEKIYO, Shigenori KAWAUCHI, Yujiro HIRAI, Toshiaki TAKEICHI, Keiko MIYA, Takashi INOMOTO, Shiro SAITO (1st Dept. Int. Med., Sch. Med., Tokushima Univ., Tokushima) **and Kazuo MIYOSHI** (Okinaka Memorial Inst. Med. Res., Tokyo)

We found 8 cases over 3 generations in one family whose plasma fibronectin (Fn) concentration was about half the normal. The proband, 31-year-old woman, showed keloid formation after burn of right upper limb, operations for appendicitis, *etc.* On examination her development was normal and there were no hyperextensibility of the skin or the joint and no abnormal susceptibility to infections. Her plasma Fn concentration was 10 mg/

dl, which is about half the normal (Laurell's method, 32 ± 6 mg/dl). Crossed immunoelectrophoresis of her Fn revealed normal pattern of mobilities. Serum and urinary hydroxyproline were both normal. Following tests of hemostasis were normal; bleeding time (4.5 min), prothrombin time (11.6 sec), KPTT (38.8 sec), and fibrinogen (243 mg/dl by thrombin time method and 237 mg/dl by Laurell's method). Cross-linking of fibrin monomers was also normal. The activity of factor XIII was normal (93%) and platelet retention and aggregation were also normal. Plasma Fn level of her father was normal (41 mg/dl) but that of her mother was decreased to 15 mg/dl, about half the normal. Decreased levels (11–17 mg/dl) were found in her two uncles, two brothers, a daughter, and a nephew. Thus, 8 of 12 family members examined showed decreased plasma Fn levels with about half the normal. None of these members, except the proband, showed symptoms related to the decreased Fn. This is a family of hereditary plasma fibronectin deficiency. The mode of inheritance is considered to be autosomal recessive and 8 individuals with decreased levels of plasma Fn are supposed to be heterozygotes. This is the first report in the world of a family with hereditary plasma fibronectin deficiency.

I-32. Genetic Polymorphism of Apolipoprotein E and Hyperlipidemia in Japanese. I. Gene Frequencies of Apolipoprotein E: Yasuko YAMANOUCHI,[1] Shigeru TSUCHIYA,[1] Ryunosuke MIYAZAKI[2] and Hideo HAMAGUCHI[1] ([1]Dept. Hum. Genet. Univ., Tsukuba, Ibaraki, [2]Dept. Med., Kudanzaka Hosp., Tokyo)

Apolipoprotein E (Apo E) phenotyping by two-dimensional gel electrophoresis (two-DE) was performed on a total of 193 apparently healthy unrelated Japanese individuals. Five Apo E phenotypes (E-3/3, E-3/4, E-2/3, E-4/4 and E-2/4) were observed. The gene frequencies of *ε3*, *ε4* and *ε2* were 0.850, 0.113 and 0.037, respectively. The incidence of hyperlipidemias with elevation of cholesterol (>250 mg/dl) or triglyceride (>160 mg/dl), or both in serum of fasting blood in adult males was significantly higher in individuals with *ε4* than in individuals with *ε3*/*ε3* ($p<0.05$). The data indicate that genetic polymorphism of Apo E is also present in Japanese and that *ε4* is associated with hyperlipidemia. The frequency of *ε2* seems to be lower in Japanese than in Caucasians.

I-33. Genetic Heterogeneity in the Human Parotid Salivary Acid Phosphatase (s-AcP) Detected by Isoelectric Focusing Electrophoresis: Shigenori IKEMOTO and Hiroshi HINOHARA (Lab. Hum. Biol., Jichi Med. Sch., Tochigi)

Genetic polymorphism of the human parotid salivary acid phosphatase (s-AcP) in Japanese population has been described, by the use of polyacrylamide gel isoelectric focusing electrophoresis with pH range 4.0–6.5. Samples of parotid saliva were collected in glass test tube with double-chamber cup of the Curby type. The parotid saliva was desalted

by dialysis at 4°C overnight. After lyophilization, samples were prepared for electrophoresis. Electrophoresis patterns of parotid salivary acid phosphatase were classified into three phenotypes. The observed phenotypes were interpreted to be controlled by two codominant alleles at a single autosomal locus. They were designated *s-Acp:A* and *s-AcP:a* and gene frequencies calculated from 183 Japanese subjects were $s\text{-}AcP:A=0.2268\pm 0.022$ and $s\text{-}AcP:a=0.7732\pm 0.022$, respectively. Since parotid saliva was used, the influence of food and oral bacteria was avoided and reproducibility was excellent. The distribution of phenotypes fitted the Hardy-Weinberg equilibrium. The data indicate that the isoelectric focusing electrophoresis is suitable for the study of heterogeneity of human parotid salivary acid phosphatase.

I-34. Amino Acid Sequence of an Amyloid Fibril Protein in Familial Amyloid Polyneuropathy of Japanese Origin: Tomotaka SHINODA, Fuyuki KAMETANI, Hiroshi TONOIKE (Dept. Chem., Tokyo Metropol. Univ., Tokyo) **and Shozo KITO** (Dept. Med., Hiroshima Univ., Hiroshima)

Amyloid fibril protein with a molecular weight of 8K daltons, in addition to one of 14 Kd, has been isolated from an autopsy specimen of a patient with familial amyloid polyneuropathy in a family from the Ogawa-Village focus. The protein component, extensively purified by repeated high performance liquid chromatography, has cross reactivity with an antiserum against normal human plasma prealbumin, and consisted of 73 amino acid residues. The purified component was digested with trypsin, and the digest was then chromatographed by HPLC to isolate tryptic peptides. By this way, total of 10 distinct peptides were obtained. Amino acid sequence of these peptides was determined by manual Edman degradation technique and the PTH-amino acids obtained were identified by HPLC. The result disclosed that it had a unique sequence which corresponded to that of the residues from 6 to 78 of normal prealbumin, except for a single amino acid replacement of methionine for valine at position 30. We have recently confirmed by recombinant DNA techniques that this replacement involved a single nucleotide substitution of G for A in a valine codon. These data strongly suggest that a variant form of prealbumin with the valine-methionine replacement at position 30, or its catabolic intermediate which has been sequenced in the present study, is also in close association with the amyloid process.

I-35. A Study on the Primary Structure of Amyloid Fibril Protein in Familial Amyloid Polyneuropathy in the Arao District of Japan: M. UEJI, T. SUZUKI, S. HIGA, S. KISHIMOTO (3rd Dept. Intern. Med., Osaka Univ., Osaka), **K. TITANI, K. TAKIO** (Dept. Biochem., Univ. Washington, Seattle), **A. HAYASHI** (Osaka Med. Cent. Res. Inst. Mat. Child Health, Osaka), **Y. TAKABA** (Arao City Hosp., Kumamoto) **and A. NAKAJIMA** (Nakajima Med. Clin., Kumamoto)

The predominant amyloid fibril proteins isolated from kidneys of four patients with familial amyloid polyneuropathy (FAP) from three genealogically independent families in the Arao district of Japan have been analysed for the primary structure. Irrespective of the patient or the family, the major protein isolated consisted of some components of a prealbumin variant, in which an amino acid substitution of methionine for valine occurred at position 30, with a heterogenous N-terminus caused by some degradation of N-terminal amino acids in the prealbumin subunit. It is very likely that this prealbumin variant is involved in the etiology of this hereditary disease, rather than being a genetic polymorphism of prealbumin.

I-36. Genetical Analysis of the Pathogenesis for Creutzfeldt-Jakob Disease: Yasuo KURODA,[1] Hidetoshi KANEOKA,[1] Masaya YAMAGUCHI,[1] Hiroshi SHIBASAKI[1] and Shuji KUME[2] ([1]Dept. Intern. Med., [2]Dept. Clin. Lab., Saga Med. Sch., Saga)

Creutzfeldt-Jakob disease (CJD) is a fatal degenerative disorder of central nervous system characterized by dementia and myoclonus seizure, with periodic synchronous discharge in EEG and subacute diffuse spongiform encephalopathy. The pathogenesis of CJD is not clarified yet, however it is widely accepted that filtrable, transmissible "unconventional" viruses cause the disease. From the findings in CJD and other slow viral infections such as Kuru and Scrapie, it is considered that genetic factors play some roles on the onset of CJD. Because HLA system is thought to be one of the key genetic elements for the immune response to microorganisms, we have analyzed the HLA phenotypes of the patients with CJD. HLA phenotypes of eleven patients with definite CJD were determined with complement-dependent microcytotoxicity test using selected sera. Nylonwool-passed mononuclear cells were used for the typing of HLA class I antigens (HLA-A, B, and C), and the adherent cells for class II antigens (HLA-DR, MT and TB). Although no association of CJD with any class I antigens was demonstrated, TB 21, one of allotypes of class II antigens, and now officially named as DQw3 at the last International Histocompatibility Conference at Munich, 1984, was found in ten patients out of eleven, whereas 302 people out of 646 have TB 21 in a healthy control (relative risk, 11.4; direct p value, 0.007). Although the high rarity and mortality of CJD have been disturbing the systematic analysis

of the genetic factors for the pathogenesis of CJD, our recent data might help to disclose the complexity of the pathogenesis of slow viral infections.

I-37. A Hereditary Abnormal Antithrombin III: Antithrombin III Toyama: Kaoru TAKAHASHI, Nobuo SAKURAGAWA, Shinichi KONDO (Cent. Clin. Lab., Toyama Med. Pharm. Univ., Toyama) **and Takehiko KOIDE** (Dept. Biochem., Niigata Univ. Sch. Med., Niigata)

A family with abnormal antithrombin III (ATIII) is presented. The proband is a 23 years old female who had suffered from recurrent thrombophlebitis involving her lower extremities. Her plasma ATIII antigen concentration, progressive antithrombin activity and factor Xa inhibitory activity were within normal range. However, the heparin cofactor activity of her plasma was decreased to about 26% of normal. On crossed immunoelectrophoresis (CIE) containing heparin in the first phase agarose, patient's ATIII showed no increase in electrophoretic mobility compared to the conventional CIE, suggesting that patient's ATIII had no affinity for heparin. From CIE pattern in the presence of heparin in the first phase agarose and the values of heparin cofactor activity, the proband was found to be a homozygote born by the heterozygous parents. The amino acid sequence analysis of this abnormal ATIII indicated that the arginine-47 of normal ATIII had been replaced by cysteine in ATIII Toyama. One base mutation, C→T in the arginine-47 genetic codon (CGT) is supposed to be responsible for this substitution.

I-38. Studies on Complement Component Deficiencies in Blood Donors in Osaka (III): Yasuo FUKUMORI, Keiji YOSHIMURA, 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)

Incidence of complement (C) component deficiencies in blood donors in Osaka has been investigated by the screening test of the hemolytic C activity in sucrose gelatin veronal buffer. Previously, we reported that two donors with C6 deficiency (C6D), 5 with C7 deficiency, 2 with C8 deficiency, 100 with C9 deficiency, and two with possible C5 deficiency (C5D) were found in 100,802 donors. In this report, the further studies on C6D and C5D individuals are described. The two donors with C6D, Y.N. (36-year-old female) and M.N. (31-year-old male), had neither personal histories of any infections nor immunocomplex disease. Both of their sera showed negligible CH_{50} titers and C6 could not be detected by hemolytic and immunochemical analysis but the other C components were in the normal range. The Y.N. family studies showed that her sister was also C6D. Parents and two children of the proposita had half the normal amount of C6. In M.N. family,

his parents also had half the normal amount of C6. These results indicate the presence of homozygotes and heterozygotes of C6D gene in the families of C6D. One of two C5D, T.I. (39-year-old female), had low level of CH_{50} (9.8). C5 in her serum could not be detected by immunochemical analysis, but hemolytic activity of C5 was 0.1% of NHS. In T.I. family, her parents had half the normal amount of C5. From these results, T.I. is considered to be inherited C5D. CH_{50} of the other possible C5D, N.K. (32-year-old male), was 19.8. Hemolytic activity of C5 in his serum was 2.7% of NHS, but no C5 protein could be detected. Further studies including family studies are required to determine whether he is C5D or not.

I-39. Genetic Polymorphisms of Human Complement Components BF and C2 in Korean: Kyung Sook PARK (Dept. Biol., Sung-Shin Women's Univ., Seoul), **Katsushi TOKUNAGA and Keiichi OMOTO** (Dept. Anthropol., Univ. Tokyo, Tokyo)

Genetically determined polymorphisms of the factor B (BF) and the second component (C2) of human complement were investigated in 220 unrelated healthy Korean using electrophoretic methods described previously. In the BF system, three phenotypes, S, FS, and F, were observed, while no rare variants were detected. The estimated allele frequencies for *BF*S* and *BF*F* were 0.775 and 0.225, respectively. In the C2 system, one common phenotype, C, and three heterozygous types, BC, BHC, and ATC, were observed. The estimated allele frequencies for *C2*C*, *C2*B*, *C2*BH*, and *C2*AT* were 0.961, 0.018, 0.011, and 0.009, respectively. *C2*BH* and *C2*AT* were reported only in Japanese previously. Significant positive association between *C2*AT* and *BF*F*, which had also been detected in Japanese, was found. Gene frequencies in Korean were compared with those in other populations.

I-40. The Fourth Component of Complement in Japanese Population and Families: K. SUZUKI,[1] K. MATSUI,[1] T. TOYOMASU,[1] H. MATSUMOTO[1] and Y. MATSUO[2] ([1]Dept. Legal Med., [2]Dept. Blood Transfus., Osaka Med. Sch., Takatsuki)

Human fourth component of complement (C4) has been found to be highly polymorphic using agarose gel electrophoresis of neuraminidase treated plasma. This protein is encoded by two separate genes, *C4A* and *C4B*, both located between the HLA-B and HLA-D(R) loci. Its polymorphism in Japanese population and families was investigated. For detection of C4 allotypes, the treatment of plasma samples and electrophoresis were carried out according to the method proposed at the Fourth International Complement Workshop, Boston (1983). Monoclonal antibody which was kindly given by Dr. G.J. O'Neill was

used to determine C4B allotypes. In general, the C4A or C4B assignment is assisted by determining its relative activity in a C4 functional overlay. Full accordance was obtained between the two methods, monoclonal antibody and hemolytic overlay except one family case where hemolytically non-functional B5 was detected. In this study, 8 allotypes were detected at each locus. C4 haplotypes were determined by family study ($p < 0.001$). These are A3B1, A4B2 and A13BQ0 and their frequencies were estimated as follows: 0.398 for A3B1, 0.148 for A4B2 and 0.028 for A13BQ0. Two family cases with duplication at the C4B locus were found in the course of studying 46 normal Japanese families. The duplication at the C4B locus were of the same type, C4B5,21 and always associated with C4AQ0, C2C and BFS, defining thus a S C Q0 5 21 complotype. One family case with hemolytically inactive B5 was found and the propositus suffered from insulin-dependent diabetes mellitus.

I-41. An Immunoblotting Technique for Complement C6 Typing: Three New Variants: Katsushi TOKUNAGA, Noriko YAMAMURA and Keiichi OMOTO (Dept. Anthropol., Univ. Tokyo, Tokyo)

An immunoblotting method for C6 typing was developed. After isoelectric focusing in thin-layer polyacrylamide gel (pH 5–8), C6 proteins were transferred passively to nitrocellulose (without electroblotting) and then detected by a two-step enzyme immunoassay. A population sample of northeastern Japanese (Iwate Pref.) was investigated using this method. Three common and four rare allotypes were observed. The allele frequencies estimated from 495 blood donors were as follows: *C6*A* 0.423, *C6*B* 0.510, *C6*B2* 0.062, and rare alleles (*91*, *M11*, *B4*, and *B5*) 0.005. Three variants, *91*, *M11*, and *B5*, were considered to be newly found. Three common and nine rare variants have been observed thus far in Japanese, of which seven (*A21*, *91*, *M1*, *M11*, *M2*, *B4*, and *B5*) were new ones. This method could be applicable to many other protein systems.

I-42. Structural Studies of a Human Gamma-3 Myeloma Protein (Kam) Bearing the Allotypic Marker Gm(st): Hideo MATSUMOTO, Shigenori ITO, Tokiko MIYAZAKI (Dept. Legal Med., Osaka Med. Sch., Takatsuki) **and Toshiyuki OHTA** (Dept. Int. Med., Occup. Environ. Health Univ., Kitakyushu)

We confirmed the amino acid substitutions determining G3m(s) (histidine at position 435 instead of arginine) and G3m(t) (methionine at position 379 instead of valine) (Matsumoto, H. *et al.* (1983) *J. Immunol.* **131**: 1865–1870), by sequence analysis of pFc′ fragment from myeloma protein (Kam) bearing the allotypic marker Gm(st). This protein was bound to Protein A-Sepharose CL-4B. The amino acid compositions and the retention times on FPLC (fast protein liquid chromatography) of tryptic peptides of carboxymethylated pFc′ (Kam) were the same as those of pFc′ (Jir). Gm(s) activity, which was exam-

ined by hemagglutination inhibition test, was disappeared by photoxidation of histidine, and Gm(s, t) were disappeared by iodination of tyrosine. Gm(s) was affected by the protein A interaction, but Gm(t) was not affected. We speculated the binding sites of Gm(s) and Gm(t) by these results.

I-43. A Unique γ_1 Heavy Chain Disease Protein Consisting of V and CH_3 Domain: **Akihiko HOSHI, Tomotaka SHINODA** (Dept. Chem., Tokyo Metropol. Univ., Tokyo), **Ikunosuke SAKURABAYASHI** (Jichi Med. Coll., Tochigi), **Kunihito IWABUCHI** (Seiwa Hosp., Tokiwagi) **and Chuichi ITO** (Iwate Med. Coll., Iwate)

Low molecular weight γ_1 heavy chain disease protein has been isolated from the serum of a patient with multiple myeloma in the following manners: 1) ammonium sulfate fractionation; 2) DEAE-Sepharose CL-6B column chromatography; 3) gel chromatography with Sephadex G-100; 4) reverse phase HPLC. The specimen (NIG-91) with a molecular weight of 22 K daltons, reacted only with an anti-human γ_1 chain. It has blocked N-terminus and glycine at the C-terminus. For sequence analyses, it has digested with TPCK-trypsin after citraconylation following complete reduction and aminoethylation. The digest was then chromatographed by reverse phase HPLC to isolate tryptic peptides. Amino acid sequence of these peptides were determined by manual Edman degradation technique and the PTH-amino acids obtained were identified by HPLC. The result disclosed that NIG-91 had 219 amino acid residues consisted of the variable domain of VHII subgroup and the constant domain of CH_3, which has allotype Glm(a) and Glm(x).

I-44. Immunohistochemistry of Human Fetuses.—Ig G, A and M Cells in the Thymus and Spleen—: **Yukio SATOW, Naotaka AKIMOTO, Juing-Yi LEE, Hiroshi SUMIDA and Naomasa OKAMOTO** (Dept. Geneticopathol. Res. Inst. Nucl. Med. Biol., Hiroshima Univ., Hiroshima)

We have been working on autopsy of normal and abnormal human fetuses and newborns to make clear the mechanism of abnormal development. Among the artificially aborted fetuses, we considered those with no abnormal findings by autopsy to be normal control cases. In this study, the Ig G, A and M cells in the thymus and spleen of nine normal human fetuses in the gestation periods of 4, 6, 7, 8 and 10 months were demonstrated by means of Avidin Biotin Peroxidase Complex method for the purpose of studying the development and differentiation of immunological activities of human fetuses and to use them as control for the purpose of making a comparison between the normal and abnormal development of human fetuses. The findings are as follows: (1) The Ig G cells appear in the thymus about 4 months of gestation and Ig A and M cells about 6 months of gestation in the thymus and the spleen in human fetal specimens. (2) The Ig G cells are seen more

often than Ig A and M cells both in the thymus and in the spleen. (3) Ig G, A and M cells are observed mainly in the medulla of the thymus and in the red pulp of the spleen mostly around the capillary vessels.

II-1. Characterization of DQw3 Alloantisera by an $F(ab')_2$ Blocking Study: **Hiroshi KUNIKANE, Tsuguyo NAKAYAMA, Shuichi HOKIN, Masanori KASAHARA, Akemi WAKISAKA and Miki AIZAWA** (Dept. Pathol., Hokkaido Univ., Sch. Med., Sapporo)

HLA-DQ antigen is one of the human major histocompatibility complex class II antigens and three specificities DQw1, w2, and w3 were officially recognized at the 9th International Histocompatibility Workshop (1984, München). The extremely strong linkage disequilibrium in Dw15-DR4-DQ blank is observed in the Japanese population. However, some of the antisera usually used as anti-DQw3 also react with Dw15-DR4 positive panels, confusing the definition of DQw3 specificity in Japanese. In order to clarify the character of those antisera, an $F(ab')_2$ blocking study was performed. Lymphoid cells from a total of 14 HLA-D/DR/DQ homozygous human B cell lines were pretreated with the $F(ab')_2$ fragment of HU-20 (anti-all DR but DR 7), and were then tested for their reactivity against those antisera. The cytotoxic activity of those antisera was eliminated or reduced with cell lines, EBV-Wa and L-KT9 (both Dw15, DR4, and DQ blank homozygous) by the pretreatment, but was unaffected for all DQw3 positive cell lines. This result indicates that those "so called DQw3 antisera" actually consisted of an anti-DQw3 component and an anti-DR4 one. Thus the problem as to DQw3 specificity in Japanese is solved.

II-2. A New HLA-DQ Specificity Recognized by a Monoclonal Antibody and Cytotoxic T Cell Clone: **Hiroshi KOJIMA, Yu'ichiro FUKASAWA, Naoshi ISHIKAWA, Tsuguyo NAKAYAMA, Hitoshi IKEDA, Akemi WAKISAKA and Miki AIZAWA** (Dept. Pathol., Hokkaido Univ., Sch. Med., Sapporo)

At the 9th International Histocompatibility Workshop, three HLA-DQ specificities, DQw1, w2 and w3 were officially recognized, although ones associated with Dw15-DR4 and Dw8-DRw8 had not been clearly defined. In order to clarify these unknown HLA-DQ specificities, a monoclonal antibody termed HU-46 was produced by immunizing C3H mouse with B lymphoblastoid cell line EBV-Wa (Dw15; DR4; DQ blank homozygous). The monoclonal antibody HU-46 was demonstrated to detect HLA-DQ antigen by two dimensional gel electrophoresis and partial amino acid sequence analyses. This monoclonal antibody specifically reacted with Dw15-DR4 positive or certain Dw8-DRw8 positive cells, whose HLA-DQ antigens had not been defined by conventional alloantisera. In addition, T cell clones were generated from mixed lymphocyte culture between peripheral blood lymphocytes from a healthy donor (DR4,6J; DQw1,w3) and irradiated EBV-Wa. Clones were screened for cytotoxic activity by ^{51}Cr release assay and cytotoxic T cell clone OW-B6 was obtained. OW-B6 recognized HLA-DQ antigen, because its cytotoxic activity was inhibited by the monoclonal antibody HU-46. Furthermore, OW-B6 showed cytotoxic

activity only against Dw15-DR4 positive or some of Dw8-DRw8 positive cells whose HLA-DQ antigens had not been defined by conventional alloantisera. These observations indicate, firstly, that HLA-DQ antigens associated with Dw15-DR4 or some of Dw8-DRw8 really exist on certain cell surfaces and display allo-activity; secondly, that they share a common determinant for a monoclonal antibody and cytotoxic T lymphocytes; and thirdly, that they may possibly constitute a new cluster as a fourth HLA-DQ specificity, HLA-DQWa.

II-3. Analysis of the Human Major Histocompatibility Complex (MHC) Class II Antigen Systems Using Two-Dimensional Gel Electrophoresis: Toshio YABE, Manabu SUZUKI, Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki), **Kazumasa MATSUKI, Tohoru NAOHARA and Takeo JUJI** (Blood Transfus. Serv., Tokyo Women's Med. Coll., Tokyo)

The MHC class II antigens are mapped on the HLA-D/DR region of the short arm of the No. 6 chromosome in human and thought to be the products of the immune response (Ir) genes. This study was performed to clarify how many kinds of human class II antigens are expressed on one D/DR-homozygous B cell line. Membrane glycoproteins of the D/DR-homozygous B lymphoblastoid cell lines were purified and labeled with ^{125}I by chloramine T method. Each class II antigens were isolated by the immunoprecipitation using mouse monoclonal antibody or alloantiserum and analyzed by two-dimensional gel electrophoresis. The DP(SB, FA), DQ(MB, DC), DR, and MT3(DRw53) antigens could be identified from each of the DR7 or DR4 cell lines analyzed. Four kinds of class II antigens were also detected from the DRw9 or DRw12 cell lines. The light chains of four kinds of antigens had different electrophoretic mobilities from one another. The heavy chains of the DP, DQ and DR antigens also differed from one another in the electrophoretic mobility. In addition, the heterogeneity of the electrophoretic mobility among different specificities was observed in each of that DP light, DQ heavy, DQ light, and MT-like light chains. These data indicate that there are at least four kinds of human class II antigen systems, DP, DQ, DR and MT and that at least seven class II genes are expressed in human. The data also suggest that genetic polymorphisms are present in at least five MHC class II gene products.

II-4. Analysis of the HLA Class II Molecules from HLA-Dw2 and Dw12: Identification and Characterization of a Novel Class II Molecule: Kikuo TSUKAMOTO, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The molecular composition of class II antigens from HLA-Dw2 and Dw12 were characterized by using 2D-Gel technique. Immunoprecipitates from Dw2 and Dw12 with anti-DR framework monoclonal antibodies (MoAbs), L-203 or HU-4 consisted of an α chain (α^1: M.W. 32–34 K) and two β chains (β^1: M.W. 28.5–30.5 K, β^2: M.W. 28–30 K). The α^1 chain and the β^1 chain from Dw12 were identical with those from Dw2, whereas the β^2 chain from Dw12 was more basic than that from Dw2 suggesting Dw2 and Dw12 differ in β^2 molecule. Anti-DQ MoAbs, SDR4.1 or Tu22 mainly precipitated an α chain (α^2: M.W. 31–33 K) and a β chain (β^3: M.W. 27–29 K). The α^2 chain of Dw12 differed from that of Dw2 in charge, and the β^3 chain of Dw12 was more acidic and had higher molecular weight than that of Dw2. Anti-DP MoAb (anti-FA) precipitated one α chain (α^2) and one β chain (β^4: M.W. 26 K). The β^4 chain from Dw12 was identical with that from Dw2, and was distinct from β^1, β^2 or β^3 chain of DR or DQ molecules. HU-4 completely inhibited the primary MLR between Dw2 and Dw12, however, anti DR2 MoAb HU-30 that precipitated only $\alpha^1\beta^1$ molecule showed no effect on the MLR between Dw2 and Dw12. Moreover, T cell proliferative response to streptococcal cell wall (SCW) antigen with antigen presenting cell was completely inhibited by HU-4 but partially by HU-30. The $\alpha^1\beta^2$ molecule is a novel class II antigen distinct from DR, DQ or DP. The MLR between Dw2 and Dw12 is stimulated only by the $\alpha^1\beta^2$ molecule and this $\alpha^1\beta^2$ molecule is recognized by T cells as a restriction molecule at the antigen presentation of SCW antigen from antigen presenting cells.

II-5. Structural Analysis of cDNA Clones Encoding HLA Class II β Polypeptides Expressed on HLA-Dw12: Kikuo TSUKAMOTO and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

We isolated several cDNA clones corresponding to HLA class II β polypeptides from cDNA library constructed from a HLA-Dw12 homozygous B lymphoblastoid cell line, EB-AKIBA using synthetic oligonucleotides as probes. These clones could be grouped into two subsets, encoding DR β or DQ β polypeptide chains. Amino acid sequence of the HLA-DQw1 β polypeptide was deduced from nucleotide sequence analysis of DQw1 β cDNA clone, pDCβ-101. This clone encoded full length DQw1 polypeptide chain. It defined a putative signal sequence, two extracellular domains, a transmembrane region and cytoplasmic tail. The transcriptionally important DNA sequence complimentary to 3′ end of 18s rRNA were expected at about 30 bp upstream from the initiation codon of

translation. A comparison of amino acid sequences of DQw1 β polypeptides from Dw2 and Dw12, amino acid replacement were predominantly located in the first domain. Partial nucleotide sequence of DR β cDNA clone, pDRβ-102 was also determined. Amino acid sequence of DR β polypeptide of Dw12 was differ from Dw2 at some positions. We characterized class II molecules expressed on Dw2 and Dw12 by 2D-Gel analysis. DQw1 molecules from Dw2 and Dw12 were differ in both α and β chains. Polymorphism of DQw1 β molecules related to DR2 haplotype was clarified on the level of amino acid sequence of β polypeptides. DR like β polypeptides from Dw2 or Dw12 consist of two β chains (β^1 and β^2). The cDNA clone encoding DR like β polypeptide, pDRβ-102 corresponding to β^1 or β^2 polypeptide is undefined.

II-6. Analysis of the Polymorphism of HLA Class II Gene Family: Kikuo TSUKAMOTO, Michio YASUNAMI and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

HLA class II genes are generally looking on as a multi-gene family. However, its detail is still undefined on the level of DNA. To investigate the gene organization and functional expression of HLA class II genes, we isolated several lambda phage genomic clones encoding HLA class II β polypeptides from human gene library using DQw1 β cDNA clone, pDCβ-101 as a probe. The structural analysis of these clones was performed by restriction endonuclease mapping and Southern blott hybridization technique using appropriate probes. Three DR β genes and two DQ β genes were identified. A series of overlapping clones encoded DQ like β and α chains. The DQ like α chain gene was located about 8 kb distant from DQ β chain gene. Moreover, these clones showed some polymorphic restriction sites in β chain coding and surrounding regions. The Southern blott hybridization analysis of high molecular weight DNA from HLA homozygous lymphocyte was performed using DR β and DQ β cDNA clones as a probe. Restriction polymorphisms of DR β genes corresponding to DR specificity were characterized using several restriction endonucleases (EcoRI, PstI, PvuII and HindIII). However, polymorphism relating to D specificity was not defined by the use of the DR β probe. Using pDCβ-101 encoding DQw1 polypeptide chain as a probe for specific to DQ β genes, DQw1 β and DQw3 β gene polymorphisms were well defined by genomic Southern blott hybridization (EcoRI, PstI, PvuII and BglII). We demonstrated the polymorphisms of class II antigens from Dw2 and Dw12 in accompanying paper. Thus diversity and polymorphisms of HLA class II genes were characterized on the level of proteins, amino acid sequences and DNA sequences.

II-7. Sialidosis (normosomatic type) の 3 同胞例と家族検索：中村 浩・高橋幸利・祐川和子・折居忠夫 (岐阜大・小児)・**大城 隆・平山清武・内藤 誠** (琉球大・医・小児，眼科)．**Sialidosis of Normosomatic Type: Clinical and Biochemical Findings in Three Affected Siblings and Their Family Members:** Y. NAKAMURA, Y. TAKAHASHI, K. SUKEGAWA, T. ORII (Dept. Pediatr., Gifu Univ., Gifu), T. OHSHIRO,[1] K. HIRAYAMA[1] and M. NAITO[2] ([1]Dept. Pediatr., [2]Dept. Ophthal., Ryukyu Univ., Okinawa)

目的：Sialidosis (α-*N*-acetylneuraminidase deficiency) は，normosomatic な type 1 と dysmorphic な type 2 に分けて考えられている．前者は Warner および O'Brien (1983) によれば，約 20 例が報告されており 15 名はイタリア人である．演者らは，17 歳女子を発端者として，同胞 4 人中 3 人に α-*N*-acetylneuraminidase deficiency が見いだされた 1 家系について報告する．

症例：症例 1) 発端者 (17 歳長女)：cherry red spots, 軽度のミオクロニー，動揺性歩行を認めたが，粗な顔貌，知能障害，視力低下，骨変化，角膜混濁，末梢血リンパ球の空胞，肝脾腫，企画振戦を認めない．症例 2) 16 歳長男：cherry red spots を有する以外異常所見をみなかった．症例 3) 9 歳次女：cherry red spots 以外に EEG 異常を認めたが，他の神経症状等特記すべきものはなかった．なお，両親は血族結婚ではない．しかし，両親にも cherry red spots がみられた．

結果：3 名の同胞に *N*-acetylneuraminidase 活性の著しい低下を認めた．両親はともに対照と患児の中間値を示した．β-galactosidase 活性は，6 名の家族全員正常域にあった．直腸粘膜の電顕で，直腸粘膜固有層内の線維芽細胞および組織球に網状顆粒状物質を含む空胞を，また組織球の一部に電子密度の高い小体が観察された．尿中に増量を認めた非還元末端にシアル酸を有するオリゴ糖画分の Bio-gel による分析は，発端者では，5 種の異なる単糖配列構造のオリゴ糖が同定されたが，両親ではシアル酸含有オリゴ糖を検出しなかった．今後さらに検索をする予定である．

II-8. A Infantile Sialidosis Associated with Congenital Adrenal Hyperplasia: Linkage between HLA Loci and the Gene for Neuraminidase Deficiency: Takahiko OOHIRA, Noriyuki NAGATA, Izumi AKABOSHI, Ichiro MATSUDA (Dept. Pediatr., Kumamoto Univ., Kumamoto), **Jiro YAMAMOTO** (Dept. Pediatr., Nobeoka Hosp., Miyazaki) **and Setsuya NAITO** (Dept. Med., Fukuoka Univ., Fukuoka)

We studied a relationship of linkage between HLA loci and the gene for neuraminidase deficiency using a female patient with combined abnormalities of congenital adrenal hyperplasia caused by 21-hydroxylase deficiency and infantile sialidosis, and her 6 family members. In her family history, consanguineous marriage was observed twice and her parents were a distant relative between each other. The patient's clinical and laboratory findings were similar to those of reported cases, including decreased neuraminidase activity which was 1% of control in cultured skin fibroblasts and 11% of control in peripheral lymphocytes. She has homozygous HLA haplotype, CW-3, TS-1, DRW9. Four of six family members examined were heterozygous for this HLA complex and their neuraminidase activities in skin fibroblasts and/or lymphocytes were intermediate between the values of the

patient and controls (25–45%), suggesting that they are carriers for sialidosis. The other two members showed normal enzyme activity and different HLA genotype. This finding suggests that the gene for neuraminidase deficiency is closely linked to HLA complex and located on chromosome 6, as the gene for 21-hydroxylase deficiency.

II-9. Spina Bifida and HLA: Koji AOKI, Satoshi FUJISAWA, Yoshiaki YAGAMI (Dept. Obstet. Gynaec., Nagoya City Univ., Nagoya), **Andrew C.W. WONG and Yoshihiko AKAHOSHI** (Dept. Orthop., Gifu Univ., Gifu)

Studies of malformations of the neuraxis and the possible relationship between them and the human major histocompatibility complex have been contributive to the search for a human equivalent of the murine T/t complex. We conducted a population and family study using 34 Japanese couples together with their offspring with neural tube defects in Central Japan. HLA-DR and DQ materno-paternal compatibility was significantly higher than the controls. Frequencies of HLA-A 11 (husband), B 35 (wife) and A 11 (affected offspring) were significantly higher than the controls. Increase in HLA-DR homozygosity among affected offspring was statistically significant. Difference in haplotypes did not reach a singificant level adequate for debate. These data are consistent and may reflect the involvement of a recessive HLA-associated gene, which in its homozygous form, may be lethal, and triggers neuraxial malformations. This further strengthened the hypothesized contribution of a T/t-like complex in or near the HLA complex to neural tube defects.

II-10. Genetic Restriction between T Cells and Monocytes in the Production of IgE Specific for Cedar Pollen Antigen: Sho MATSUSHITA,[1] Masahiko MUTO,[2] Yoji TAKAHASHI,[2] Yozo SAITO[3] and Takehiko SASAZUKI[1] ([1]Dept. Genet., Med. Inst. Bioregul., Kyushu Univ, Fukuoka; [2]Dept. Hum. Genet., Med. Res. Inst., [3]Dept. Otolaryngol., Sch. Med., Tokyo Med. Dent. Univ, Tokyo)

We have reported that IgE nonresponsiveness to cedar pollen antigen (CPAg) is controlled by an HLA-linked dominant gene, and that the nonresponsiveness *in vitro* is mediated by both antigen-specific and isotype-specific suppressor T cells. In order to investigate the T-monocyte interaction in CPAg-specific IgE production, we obtained monocyte-free fractions of T and B cells using anti-Leu M3 and complement. Pokeweed mitogen (PWM)-stimulated IgE synthesis of peripheral blood lymphocytes required T cells, B cells, and monocytes. Since allogeneic monocytes equally stimulated T+B cell population to produce IgE, it was possible to use PWM in order to investigate T-allogeneic monocytes interaction in CPAg-specific IgE production. T and B cells were cocultured with allogeneic or autologous monocytes from responders, PWM, and CPAg. CPAg-specific IgE in culture supernatant were assayed using solid-phase RIA. The same level of CPAg-specific

IgE was detectable as in autologous combination only when monocytes and T+B cell populations shared at least one HLA-DR antigen, indicating that T-monocyte interaction in CPAg-specific IgE production is restricted by HLA-DR. Furthermore, IgE synthesis specific for CPAg was blocked by monoclonal antibody to HLA-DR framework antigen (HU-4), confirming a crucial role of HLA-DR molecule in this response.

II-11. Immunogenetic Analysis of Leprosy (IV): Ikuo KIKUCHI, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Toshiharu OZAWA** (Natl. Inst. Leprosy Res., Tokyo), **Kiyotaka SANADA, Masanori KOSEKI** (Natl. Sanat. Tama-zenshoen, Tokyo) **and Shigeru KUMAMARU** (Natl. Sanat. Kikuchi-keifuen, Kumamoto)

We have reported that an HLA-linked gene controlls the clinical manifestation of leprosy through T lymphocytes. In this paper, we have investigated the genetic restriction of the immune response to *M. leprae* antigen. The immune response to *M. leprae* antigen of peripheral blood lymphocytes from patients with tuberculoid leprosy was completely blocked by anti HLA-DR framework monoclonal antibody (MoAb). *M. leprae* specific T cell line from patients with tuberculoid leprosy were established using Interleukin-2 prepared from the culture supernatant of the Gibbon lymphoma MLA-144. The T cell lines showed the proliferative response specific to *M. leprae* antigen in the presence of allogeneic Mϕ which shared at least one HLA-DR antigen with T cell donors. The allogeneic T cell line-Mϕ interaction was completely blocked by anti HLA-DR framework MoAb. From these data, we concluded that the genetic restriction by HLA-DR antigen exists in the immune response to *M. leprae* antigen. Furthermore, the restoration of the immune response of a patient with lepromatous leprosy (non responder) to *M. leprae* antigen was observed by adding anti HLA-DP MoAb. This observation suggested that the HLA-DP antigen might be a restriction molecule for the non responsiveness to *M. leprae* antigen in patients with lepromatous leprosy.

II-12. Genetic Control of Immune Response: Kenji HIRAYAMA, Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

Genes in the HLA-D region control the immune responsiveness to natural antigens and the susceptibility to certain diseases. So it is important to analyse the function of their gene products, HLA-class II molecules. We have already reported that new DR like class II molecule DT is responsible for mixed lymphocyte culture reaction (MLR) between Dw2 and Dw12 which are serologically typed DR2 and that DR β-chains of DR4 associated with HLA-D haplotypes, Dw4, Dw15 and DKT2 are different from one another in two

dimensional polyacrylamide gel electrophoresis profiles. In this study, T cell lines specific for streptococcal cell wall (SCW) antigen were established from HLA-Dw12/D-blank, HLA-Dw4/DKT2 and HLA-Dw15/D-blank heterozygote. All of the T cell donors were high responders to SCW. DR4 positive T cell lines proliferated in the presence of HLA-D shared allogeneic antigen presenting cells (APC) and antigen. Even if APC shared HLA-DR4, HLA-D non-shared APC did not present SCW antigen at all. So these T cell lines established from DR4 positive donors recognized the polymorphism of β-chain of DR4 molecule. Dw12 positive T cell line showed marked immune response in the presence of Dw12 sharing APC. Decreased but apparently positive immune response was observed in the presence of Dw2 positive APC. HLA-DR non-shared APC did not present antigen at all. These T cell lines-APC cooperation was completely abolished by anti HLA-DR framework monoclonal antibody HU-4. Both DR and DT molecule played a role of restriction molecule in the cooperation between this T cell line and APC. It is suggested that HLA linked immune response gene controls the immune response to SCW through these class II molecules.

II-13. Immunogenetics of Adult T-Cell Leukemia (1): Hisamitsu UNO, Kiyohide KAWANO, Nobuyoshi TACHIBANA and Kazunori TSUDA (2nd Dept. Med., Miyazaki Med. Coll., Miyazaki)

Adult T-cell leukemia (ATL) is a unique T-cell malignancy caused by human T-cell retrovirus (HTLV). We investigated the role of HLA, human major histocompatibility complex (MHC), in pathogenesis of ATL, since it has been shown that genes linked to H-2, murine MHC, control susceptibility to leukemias caused by retroviruses. We investigated 39 patients with ATL, 20 patients with T-cell malignant lymphoma (T-ML) and 35 healthy carriers who were positive for antibodies against ATL-associated antigens (anti-ATLA antibodies). Based on clinical features patients with ATL were divided into three groups; 30 patients with acute ATL, 6 patients with chronic ATL and 3 smoldering ATL. T-ML was tentatively defined as histologically diagnosed non-leukemic lymphoma, regardless of the presence of anti-ATLA antibodies. Class 1 HLA antigens showed altered expression in acute ATL; either extra HLA antigens or decreased expression of the antigens in proportion to the number of leukemic cells in peripheral blood. HLA-B5 cross reactive group antigens showed significant difference between acute ATL and chronic ATL, 96.7% and 0% respectively ($p < 5 \times 10^{-6}$). HLA-Bw62 showed significant association with acute ATL ($p < 2 \times 10^{-4}$). HLA-B7 was absent in both ATL and T-ML. Class 1 HLA showed no association with healthy carriers. Analysis of anti-ATLA antibodies in 15 informative families showed that HLA haplotypes did not segregate with positivity of the antibodies. Furthermore, HLA showed no association with positivity or negativity of the antibodies

in 30 couples where either or both of spouses were positive for the antibodies. The results indicated that HLA exerts an influence on clinical course of ATL, but not on infection of HTLV.

II-14. Chromosome Changes in Gynecologic Cancer: Nobuyoshi OZAWA, Kan-ichi SOH, Toshifumi TAKABAYASHI, Soujin SOU, Kiyo SASAMOTO, Noriko MIYASHITA, Shigeki UEHARA, Akira YAJIMA and Masakuni SUZUKI (Dept. Obstet. Gynec., Tohoku Univ. Sch. Med., Sendai)

Since the discovery of Ph^1 chromosome, some specific chromosomal abnormalities in certain leukemias have been reported, but the chromosomal analysis of solid tumors has not been done as well because of its technical difficulty. In this report, we present the results of chromosomal analyses in three ascites tumors and nine solid tumors.

1) Case 1: epidermoid carcinoma of ovary (ascites), modal chromosome number (MCN) =47. Case 2: ovarian carcinoma (ascites), MCN=46. Case 3: Kurkenberg's tumor (ascites), MCN=42. Case 4: dysgerminoma, MCN=86. Case 5: uterine sarcoma, MCN =63 and 74. Case 6: endometrial carcinoma (Virchow lymphnode), MCN=75. Case 7: endometrial carcinoma (G_1), MCN=46. Case 8: endometrial carcinoma (G_3), MCN= 95. Case 9: adenomatous hyperplasia, MCN=46. Case 10: endometrial carcinoma (G_1), MCN=46. Case 11: endometrial carcinoma (G_1), MCN=45. Case 12: endometrial carcinoma (G_2), MCN=59. 2) By karyotypic analyses, an extra copy of a part of the long arm of chromosome #1 was commonly found in cases 1, 2, 3, 4, 6 and 8. 3) The following characteristic marker chromosomes were discernible: a large marker due to t(1;11)(q21; q35) in case 1, a large marker due to t(1:4)(q11;q23) in case 3, and 7q+ in cases 3 and 12.

II-15. Chromosome Abnormalities in Down's Syndrome Patients with Acute Leukemia: Yasuhiko KANEKO, Nobuo MASEKI, Masaharu SAKURAI (Saitama Cancer Cent., Saitama), **Tatsuto SUZUKI** (Dept. Pediatr., Kainan Hosp., Aichi), **Ryo KOIDE** (Natl. Children's Hosp., Tokyo) **and Shinpei NAKAZAWA** (Dept. Pediatr., Keio Univ., Tokyo)

Chromosome and cytologic studies were performed on 5 Down's syndrome (DS) patients with acute nonlymphocytic leukemia (ANLL). All 5 patients had an aneuploid clone in their leukemic cells: Case 1. 48,XY,+8,+21; Case 2. 48,XX,+8,+21; Case 3. 47,XY,+8, −21,+dic(21;21)(p13;p11); Case 4. 52,XX,+8,+14,+19,+21,+21,+21; Case 5. 50,XX, +6,+19,+21,+22. Every patient appeared to have acute undifferentiated leukemia when the blast cells were examined with Wright-Giemsa stain; cytochemistry studies, however, showed that the leukemic blasts in Cases 2, 3, and 5 were in early stage of myeloid differentiation, and electron microscopic study on Case 4 led the diagnosis of megakaryoblastic

leukemia. We found 7 other cases with DS and ANLL in literature; 2 of them had a karyotype of 48,XX,+8,+21, and 2 had many extra chromosomes including +19 and two +21s in their leukemic cells. Our findings and a review of data on 7 other patients showed that numerical changes including +8, +19, and/or +21 are quite common in DS ANLL and that these changes may be associated with leukemic cells in an early stage of myeloid differentiation.

II-16. Cytogenetic Studies in Myelodysplastic Syndrome: Relation of Initial Karyotype and Karyotypic Evolution to Leukemic Transformation: Masafumi TANIWAKI, Shigeo HORIIKE, Kazuhiro NISHIDA, Shoichiro TSUDA, Taira MAEKAWA, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (Dept. Med., Kyoto Pref. Univ. Med., Kyoto)

Chromosomal analyses on bone marrow cells from 37 patients with myelodysplastic syndrome (MDS) were performed with various banding techniques including new methods using base specific antibiotics and fluorochromes. MDS was subclassified into 6 categories according to French-American-British classification: Refractory anemia (RA, 7 cases), RA with ring sideroblasts (2 cases), RA with excess of blasts (RAEB, 9 cases), RAEB 'in transformation' (11 cases), CMML (5 cases), and unclassified MDS (UMDS, 3 cases). Twenty five cases (68%) showed a variety of chromosome abnormalities, among which the structural rearrangements involving the long arm of No. 5 or the short arm of No. 2 were most frequent. The structural changes involving the long arm of No. 5 were observed only in cases of RAEB 'in transformation' and UMDS. Ten of 23 cases showing chromosome abnormalities and 2 of 11 cases without chromosome abnormalities developed overt leukemia. Serial cytogenetic analyses were performed on 9 cases. Five of them developed leukemia, including 2 cases with karyotypic evolution. The remaining 4 cases did not show overt leukemia nor karyotypic evolution. These results suggest that the initial karyotype and karyotypic evolution are of prognostic significance in respect of leukemic transformation of MDS.

II-17. Cytogenetic Studies on 30 Patients with Non-Hodgkin's Lymphoma: Kazuhiro NISHIDA, Masafumi TANIWAKI, Shouhei YOKOTA, Shigeo HORIIKE, Hiromi YASHIGE, Yuji OKAMOTO, Shoichiro TSUDA, Taira MAEKAWA, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (3rd Dept. Med., Kyoto Preft. Univ. Med., Kyoto)

Using G, R(CMA/DMA), C(DMA/DAPI) or Q(AMD/DAPI) banding techniques, chromosomal analyses were made on lymph node, bone marrow or spinal fluid cells from 30 patients with non-Hodgkin's lymphoma. These included 3 cases of Burkitt's lymphoma

(L.), 10 cases of B-cell L., 7 cases of T-cell L., 3 cases of SmIg negative non-T-cell L., and one case each of immunoblastic L. and CALL type L., which were all diagnosed using the international histologic formulation and immunologic markers. The remaining 5 cases of lymphomas immunologically not examined were also studied. Clonal chromosome abnormalities were detected in 29 (96%) of the 30 patients. Relatively common numerical changes were gains of chromosomes No. 3 (23% of the patients), No. 21 (20%) and No. 22 (20%). The chromosome regions frequently involved in structural changes were 1q (43% of the patients), 1p (40%), 14q (33%), 11p (27%), 6q (23%) and 11q (23%). The site most frequently involved in translocations was 14q32. There were 2 recurring translocations: one was t(8;14)(q24;q32) in 2 cases of Burkitt's L. and one case of immunoblastic sarcoma, and the other was t(7;14)(q32;q32) in 2 cases of diffuse type lymphoma. In one patient with follicular lymphoma a t(14;18)(q32.3;q21.3) was found. Other sites involved in translocations of t(14;−)(q32;−) were 1p22, 1q42.1, 2q35 and 3q27. A translocation (1;19) was found in a case of CALL type lymphoma. The same translocation was reported in patients with preB-cell leukemia (Carroll, 1984). We conclude that most of lymphomas have cytogenetic abnormalities.

II-18. Double Minutes in a Patient with CML in Blastic Phase: Mariko UEHARA,[1] Mitsushiro KIDA,[1] Hiroko TESHIROGI[2] and Masahide KAMAKURA[2] ([1]Dept. Pediatr., [2]1st Dept. Intern. Med., Teikyo Univ., Tokyo)

The patient was a 33-yr-old female with chronic myelocytic leukemia in blastic phase, who showed less response to the treatment with Busulfan and Dibromomannitol. Chromosome studies were performed on bone marrow cells cultured for one day or 4 days. The ranges of chromosome number were 30–52 and 37–50 in one-day- and 4-days-cultures, respectively. Chromosome abnormalities observed were: double Ph[1], i(17), +B, +6, +19, +r(D) and double minute chromosomes (DMs). DMs could be classified into 3 types according to their sizes: small (S), medium (M) and large (L) ones. The large DMs were occasionally seen as small ring (sr) chromosomes. Frequencies of cells with each type of DMs and rings were scored in both the one-day- and 4-days-cultures, and the results were as follows. One-day-culture: S 43%, M 62%, L 5% (sr 3%), r(D) 10%, based on a total of 70 metaphases examined. The mean numbers of DMs per cell were 6.5 for S and 2.2 for M. Four-days-culture: S 71%, M 38%, L 15% (sr 9%), r(D) 7%, based on 54 metaphases. The mean numbers of DMs per cell were 14.0 for S and 2.5 for M. The maximum numbers of DMs per cell were over 100 for S, 10 for M and 1 for L. The above findings implicate that the number of DMs of S type increased by duplication during the culture period, and advanced the following suggestions: 1) DMs of S, M and L types were

different in origin from one another. 2) The duplication rates of S-DMs is faster than M and L-DMs. 3) The growth advantage of the cells with S-DMs is higher than the cells with M- or L-DMs.

II-19. Cytogenetic Studies on Childhood Solid Tumors: Yasuhide HAYASHI, Yuji HABU, Ryoji HANADA, Keiko YAMAMOTO (Div. Hematol. Oncol., Saitama Child. Med. Cent., Saitama), **Toshiro NISHIDA** (Div. Lab., SCMC, Saitama) **and Masaharu SAKURAI** (Div. Chemoth. Saitama Cancer Cent., Saitama)

Chromosomal analysis was performed on 10 tumors and a cell line of neuroblastoma (NB), 2 tumors of rhabdomyosarcoma (RMS) and a Wilms' tumor (WT). All specimens but one NB had clonal abnormalities (abns). Among NBs 1p abns were found in 2 tumors, the breakpoints showing 1p32 and 1p34, respectively. Homogeneously staining region (HSR) was observed in a tumor of relapsed case and double minutes (DMs) in the cell line established from a case with NB who had no DMs at initial diagnosis. Hyperdiploidy (>50) was observed in 4 tumors, two of which were found by VMA screening test, and normal karyotype in a case with IVs NB. A t(2;8)(q27;q22) and 3p abn were observed in a case with RMS, respectively. Del(11)(p13) was observed in a case with WT (rhabdoid type). Recently c-*ras* oncogenes have been assigned to 1p, 3p and 11p. Our results suggest the possibility that the childhood solid tumors are associated with c-*ras* oncogene.

II-20. 絨毛上皮腫の起源：今泉　清・萩原啓二・梶井　正（山口大・小児）. **Origin of Choriocarcinoma:** Kiyoshi IMAIZUMI, Keiji HAGIWARA and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ., Yamaguchi)

材料：1) 全奇胎に続発した絨毛上皮腫 (83-CHO1) の短期培養細胞．その宿主と夫の末梢血リンパ球．2) 全奇胎に続発した肺転移のヌードマウス接種株 (CC-HM-1)．その宿主の夫のリンパ球．方法：いずれも染色体標本を作製．Q－，R－ 分染法による染色体異型をマーカーとして起源を同定した．さらに esterase D, phosphoglucomutase I と III の多型を併用した．結果：83-CHO1 はモード核型 49,X,+der(1), +3, −6, +11, +12, +der(C), +der(14), +der(15), −17, −19. centromere から遠位のアイソザイム多型 3 種のうち 2 種はヘテロ接合を呈したから，ハプロイド精子による受精後の 2 倍化は否定できる．centromere 近傍の染色体多型で情報のあった 6 組の多型は，父がヘテロ接合，絨毛上皮腫はすべてがホモ接合を呈した．したがって第 2 成熟分裂の不分離による diplospermy と推定できる．ただし，2 核精子受精の可能性は 1/64 で完全には否定できない．CC-HM-1 は，核型 48, X, +der(2), +3, +der(4), +der(7), +der(7), −7, −8, −13, −13, t(13q:15q), +der(16), +der(21), +der(21), −21, −22. 2 組の多型が分析可能で，父はヘテロ接合，絨毛上皮腫はホモ接合だった．

II-21. Cytogenetic Studies on Cultured Skin Fibroblasts from Patients with Familial Polyposis Coli and Peutz-Jeghers' Syndrome: **Setsuo TAKAI,[1] Mitsuo OSHIMURA,[1] Akira TONOMURA[1] and Takeo IWAMA[2]** ([1]Dept. Cytogenet., Med. Res. Inst., [2]2nd Dept. Surg., Fac. Med., Tokyo Med. Dent. Univ., Tokyo)

In order to examine the occurrence of cells with specific chromosome rearrangements, chromosomal instability was studied on cultured skin fibroblasts derived from patients with familial polyposis coli (FPC) and Peutz-Jeghers' syndrome (PJ). Fibroblast cultures were grown from skin biopsies obtained from 10 patients with FPC and 6 patients with PJ. After the third to sixth culture passage, chromosome analysis was made on 50 cells for each case using conventional Giemsa staining method. When cytogenetically aberrant cells were present, Q-banding technique was applied on the same slide and the aberrant cells were photographed for detailed analysis by photoprints. In the cases of FPC the cells with reciprocal translocations were found in 3 cases and the cells with inversions were in 3 cases. The cells with reciprocal translocations were detected in 3 cases of PJ. The mean frequency of cells with these structural aberrations, including terminal deletions was 3.6% for FPC and 4.0% for PJ, and some were categorized as belonging to the one clone where the identical karyotype was present in more than one cell. The chromosome rearrangements were occurred in some chromosomes; such as Nos. 2, 4, 8, 12 and 14, but No. 1 was more frequently involved as compared with other chromosomes. The breakpoints concerned in the formation of chromosome rearrangements tended to cluster to region p3 of No. 1 chromosome in both patients with FPC and PJ.

II-22. 大腸癌の細胞遺伝学的研究：越智尚子・外村　晶(東医歯大・難研・細胞遺伝)．**Avery A. Sandberg** (Roswell Park Memorial Inst., Buffalo, USA). **Cytogenetic Analysis of Primary Large Bowel Cancer:** Hisako OCHI, Akira TONOMURA (Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo) and Avery A. SANDBERG (RPMI, Buffalo, USA)

われわれは，大腸癌の染色体分析を行い，non-random な染色体異常を見いだしたので報告する．被検材料は，1982 年 2 月より 1983 年 12 月まで Roswell Park Memorial Institute において大腸癌切除を受けた患者 21 例より得た 22 個の癌組織であった．癌組織は切除後，ただちに，病理標本と染色体検査用標本に分割した．染色体検査用標本は，細切後，collagenase II を用いて細胞浮遊液を作製し，短期培養後，G-バンドないし Q-バンド法にて分析した．21 例中 19 例にクローン性の染色体異常が認められた．19 例中 7 例は，染色体数モードが 3n 域以上であった．最も多く認められた数的異常は，−18 (9 例)，+7 (7 例)，+12 (6 例) であった．構造異常は，No. 18 と Y 染色体を除く全染色体に認められ，No. 7 の関与する構造異常が最も多く (8 例)，以下 No. 1, No. 4, No. 17 (各 7 例)，No. 6, No. 11 (各 6 例) であった．さらに，上記の染色体異常と臨床像 (腫瘤の部位，分化度および stage) の関連について検討してみたが，一定の傾向は得られなかった．しかし，染色体数モードが 3n 域以上の 7 例では，6 例の腫瘤が S 状結腸部以下に存在した．近年，ヒト大

腸癌において見いだされた発癌遺伝子である k-ras-2 が 12 番目の染色体上にあることが明らかになった．われわれの検討でも No. 12 染色体に数的ないし構造異常をもつ症例が 21 例中 14 例 (67%) に認められ，発癌遺伝子の活性化と染色体異常との関連を考えるうえで興味深い．

II-23. Chromosome Localization of a Gene in Normal Cells for Suppression of Colony Forming Ability in Soft Agar of a Murine Plasmacytoma: Tsuneyuki OIKAWA and Noboru KUZUMAKI (Lab. Genet., Cancer Inst., Hokkaido Univ., Sch. Med., Sapporo)

Using hybrid clones isolated from several series of fusion between a BALB/c mouse plasmacytoma (S194·Bu^r·Oua^r) and normal spleen cells or fibroblasts of CBA/H-H6 mice, the relationship between chromosome constitution and colony forming ability in 0.33% soft agar was investigated. All the hybrid clones (10 clones) between S194 and normal spleen cells grew rapidly like parental S194 cells as a suspension form in liquid medium, and were positive for colony formation in soft agar. In chromosome constitution, these hybrid clones lost about half of normal chromosome complements, but always retained the murine plasmacytoma-specific t(12;15) chromosome where the *c-myc* oncogene is transposed. On the other hand, the hybrid clones (13 clones) between S194 and normal fibroblasts grew slowly as an attached form in liquid medium, and were divided into two groups on the basis of their colony forming ability in soft agar: 6 clones showing pile-up growth in liquid medium were positive for colony formation in soft agar, and 7 clones showing contact inhibition in liquid medium were negative for colony formation in soft agar. Chromosome analysis of these clones revealed that the hybrid clones positive for colony formation in soft agar commonly showed retention of t(12;15) chromosome, and loss of one copy of chromosome 4. The hybrid clones negative for colony formation in soft agar showed either loss of t(12;15) chromosome (2 clones), or retention of t(12;15) chromosome with 4 copies (full set) of chromosome 4 (5 clones). The hybrid clones positive for colony formation in soft agar, however, retained two copies of normal counterpart of t(12;15) chromosome; t(14;15) and T6 marker chromosomes. These results suggest that a gene in normal cells for suppression of colony forming ability of S194 cells may locate on normal chromosome 4, but not on normal chromosome 15.

II-24. Cytoskeletal F-Actin Patterns in Skin Fibroblasts from Normal Subjects of Various Ages and Patients with Autosomal Dominant Disorders Predisposed to Neoplasia: K. OHNO and K. TAKESHITA (Div. Child Neurol., Inst. Neurol. Sci., Tottori Univ., Yonago)

Formaldehyde fixed, acetone extracted cells were labelled with 7-nitrobenz-2-oxa-1,3-diazole(NBD)-Phallacidin, which has highly affinity for F-actin and does not bind monometric G-actin. Normal skin fibroblasts from 4 apparently normal fetuses, 9 children and

11 adults, fibroblasts from patients with adenomatosis of the colon and rectum (ACR) (3 strains), Recklinghausen's neurofibromatosis (NF) (4 strains), basal cell naevus syndrome (BCN) (one strain) and tuberous sclerosis (TS) (6 strains), and an SV40 transformed human skin fibroblast strain (SVLN) were examined. In SV40 transformed cells, about 75% cells show less or no organized actin fibers, as have been reported by Pollack *et al.*, 1975. In rapidly growing cultures of fibroblasts from normal subjects, less organized actin fibers were shown about 25% cells in cultures from fetus and children, whereas only 15% cells in cultures from normal adults ($p < 0.001$). Increased organization of F-actin accompanying the aging of the donors is similar to that accompanying the aging of fibroblasts *in vitro* (Wang and Gundersen, 1984). F-actin patterns of patients with autosomal dominant disorders predisposed to neoplasia were almost similar to those of normal fibroblasts, except for one strain from an ACR patient. Skin fibroblasts from patients with ACR have been reported to have less organized F-actin fibers (Kopelovitch *et al.*, 1977, 1980). Our results suggest that some of ACR patients show this abnormality, but this is not a consistent abnormality in ACR fibroblasts.

II-25. Chromosome Analysis of Cell Lines Producing Human T-Cell Leukemia Virus and Simian Related Viruses: Kumiko IIJIMA,[1] Atsumi KOMURO,[1] Hajime TSUJIMOTO,[2] Masanori HAYAMI[2] and Munehiro HIRAYAMA[1] ([1]Dept. Maternal Child Health, Sch. Health Sci., Fac. Med., [2]Dept. Animal Pathol., Inst. Med. Sci., Univ. Tokyo, Tokyo)

Adult T-cell leukemia (ATL) is a T-cell malignancy endemic in southwestern Japan, which is associated with a retrovirus named human T-cell leukemia virus (HTLV). On the other hand, retroviruses closely related to HTLV were isolated from several species of nonhuman primates. Human lymphocytes were infected *in vitro* with HTLV or simian related viruses, and their cytogenetic properties were sequentially analyzed. Human peripheral blood lymphocytes free of HTLV were co-cultivated with lethally irradiated lymphoid cell lines producing HTLV or simian retroviruses, and maintained in the presence of IL2. The expression of HTLV-related antigens was examined by immunofluorescence assay. Chromosomal anomalies and sister chromatid exchanges (SCE) were examined on these cell lines at several passage levels. Various numerical chromosomal anomalies were found in all cell lines, which were not consistent with certain chromosomes. The frequency of aneuploid cells and cells with minute chromosomes increased gradually as the passage level advanced. Double minute chromosomes were observed in all cell lines and the frequency ranged from one to more than 20. The number of SCE per chromosome ranged from 0.20 to 0.57, which was higher than that observed in normal cells.

II-26. ネオカルチノスタチンでヒト培養リンパ球に誘発される染色体異常：津田昌一郎・西田一弘・前川　平・谷脇雅史・三沢信一・阿部達生・瀧野辰郎(京都府医大・三内). **Neocarcinostatin-Induced Chromosome Aberrations in Human Lymphocyte Cultures:** Shoichiro TSUDA, Kazuhiro NISHIDA, Taira MAEKAWA, Masafumi TANIWAKI, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (3rd Dept. Intern. Med., Kyoto Pref. Univ. Med., Kyoto)

ネオカルチノスタチン (NCS) のヒト培養リンパ球の染色体に及ぼす影響を調べたところ，多彩な交換型を主とする染色体異常を認めた．健常ヒトリンパ球を RPMI1640 培地 (15% FCS) で PHA 添加のもとに培養した．NCS を 10^{-3}～10^{-11} M で培養開始時より作用させ，72 時間後および 48 時間後に標本を作製し，G および C 染色を行った．また 24 時間後に BrdU (終濃度 5 μg/ml) を加えたものでは，FPG 染色を施行した．10^{-6} および 10^{-7} M NCS を作用させたとき，染色体数は triploid から tetraploid 域に分布し，duplicated dicentrics や tricentrics および paired minute chromosome, rings など多彩な交換型異常が認められた．48 時間培養では hypodiploid から hyperdiploid 域に染色体数は分布し，同様の異常が認められたが，duplicate していることはなかった．次にカフェイン (5×10^{-3}～10^{-7} M), および AraC (10^{-7}～10^{-10} M) を培養開始時より NCS と同時に作用させた．高濃度のカフェイン処理で，ごく一部の細胞に pulverization が認められた．またほぼ等モルの AraC 同時処理では，NCS により誘発された異常が消失していた．結論：NCS により誘発された DNA の損傷はきわめて修復を受けやすく交換型の異常を形成し，かつなんらかの機序で倍加される．カフェインは NCS による損傷修復に多少の効果を示し，AraC は強く抑制することが知られた．

II-27. 21 トリソミーのリンパ球における放射線による染色体異常誘発に対する化学的防護について：斎藤深美子[1]・外村　晶[1]・松原　升[2] (東医歯大・[1]難研・細胞遺伝，[2]医・放射線). **The Effects of Scavengers to γ-Ray Induced Chromosome Aberrations in the Lymphocytes with 21 Trisomy:** Fumiko SAITO,[1] Akira TONOMURA[1] and Sho MATSUBARA[2] ([1]Dept. Cytogenet., [2]Dept. Radiol., Tokyo Med. Dent. Univ., Tokyo)

トリソミーのリンパ球は，放射線によって誘発される交換型異常の頻度が，正常のリンパ球に比べて有意に高いことが知られており，また放射線によって生体中に生ずるさまざまな活性酸素と遺伝的損傷との関係が論じられている．本研究では，トリソミーのリンパ球での放射線誘発染色体異常生成に関与する活性種を調べるため，1) ·OH, 2) O_2^-, 3) H_2O_2, および 4) $^1O_2$ に作用する各除去剤を用いてその効果を正常細胞での結果と比較検討した．その結果，放射線によって誘発される交換型染色体異常の主要な要因は，正常細胞と同じく 21 トリソミーの細胞でも，·OH であることが確認された．一方 2) や 3) の除去剤 SOD あるいは catalase は放射線の障害に対して，トリソミーの細胞では必ずしも防護的に作用するとは限らず，むしろ促進作用をもつ可能性が示唆された．また 4) の除去剤の結果から，$^1O_2$ の関与の可能性も出てきたので，さらに詳細な検討が必要であろう．

II-28. Proliferative Kinetics and Chemical-Induced Chromosomal Damages in Human Peripheral Lymphocytes Cultured in Medium without Serum: Kanehisa MORIMOTO, Mayumi MIZUNO-SATO, Kunihiko MIURA and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

Peripheral blood lymphocytes, which is now widely adopted in cytogenetical studies, are usually cultured in medium containing 10–15% fetal bovine serum (FBS). The authors have already reported the influences of various culture conditions of lymphocyte culture on the sister chromatid exchange (SCE) frequencies. In the present study, we further studied the effects of adding FBS to culture media on the cell proliferation kinetics or chemical-induced chromosomal damages (SCEs or structural aberrations). Whole blood was added to various culture media (RPM1 1640, MEM, F-10) with or without 15% FBS and then cultured with phytohemagglutinin for 72 hr. When cell proliferation kinetics was investigated in FPG-stained samples, faster proliferation kinetics were observed in cells cultured without FBS in all the culture media. On the other hand, no difference was observed in the dicentric and ring frequencies in lymphocytes cultured in RPM1 1640 medium with or without FBS after γ-irradiation (2 Gy). Finally, frequencies of SCEs induced by mitomycin C (3×10^{-8} M) were shown to be about 50% higher in cells from non-serum culture than those in cells from plus-serum culture, while baseline SCE frequencies were about the same in cells from both culture.

II-29. Effects of Various Metabolic Inhibitors on the Chromosomal Damages in Human Peripheral Lymphocytes: Kunihiko MIURA, Kanehisa MORIMOTO, Mayumi MIZUNO-SATO and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

The effects of various metabolic inhibitors on the frequencies of the dicentric and ring (D+R) chromosomes induced by γ-rays or HTO β-rays, or of the sister chromatid exchanges induced by mitomycin-C in peripheral blood lymphocytes were investigated. The inhibitors used and their final concentrations are as follows: Caffeine, 500 μg/ml; FUdR, 10 μg/ml; cycloheximide, 10 μg/ml; 3-aminobenzamide, 10 mM; hydroxyurea, 5 mM; cytosine arabinoside (ara-C), 50 μM; aphidicolin, 300 nM; and novobiocin, 50 μM. When lymphocytes were irradiated by γ-rays (2 Gy) and then treated with ara-C before PHA-stimulation, the D+R frequencies increased by about 95%. The enhancing effects of ara-C on the γ-ray-induced D+R frequencies were observed when cells were pre-treated with this agent 1–3 hr before γ-irradiation. Post-treatment of γ-irradiated cells with caffeine or FUdR also increased D+R frequencies by 30–70%. Cytosine arabinoside, caffeine, or FUdR also increased D+R frequencies in cells treated with HTO β-rays; the enhancement ratio being 4.20, 1.37, and 1.54 respectively. Furthermore, all the inhibitors tested were

shown to influence SCE frequencies or cell proliferation kinetics in cells treated with mitomycin C.

II-30. Gamma-Ray Induced Mutation in Ataxia Telangiectasia Lymphoblastoid Cells: Kouichi TATSUMI,[1] Mariko TOYODA and Hiraku TAKEBE[2] ([1]Radiat. Biol. Cent., [2]Dept. Exp. Radiol., Fac. Med., Kyoto Univ., Kyoto)

Ataxia telangiectasia (AT) is an autosomal recessive disease associated with high incidence of lymphoreticular malignancy in young adults. Although fibroblasts from AT patients are hypersensitive for cell killing by ionizing radiation, they have been reported to be hypomutable or even immutable to ionizing radiation. The use of diploid lymphoblastoid cell lines enabled us to perform mutation experiments yielding a sufficient number of surviving mutants after γ-irradiation. The AT lymphoblastoid cell line, GM2783, was found to be not immutable by γ-irradiation. The frequency of mutation as a function of dose in AT cells was comparable to that in repair proficient cells.

II-31. Cytogenetic Studies on the Offspring of A-Bomb Survivors—on the Frequency of Autosomal Structural Rearrangements in Hiroshima: Akio A. AWA, Yoshiaki KODAMA, Masashi HIRAMOTO and Sumie MURATA (RERF, Hiroshima)

Chromosome surveys on 9,427 children (4,492 exposed and 4,935 controls) have been completed by the end of 1983 in Hiroshima. This report describes the frequency of persons with autosomal structural rearrangements of balanced type among F_1 cohort, with special reference to the mutation rate on these abnormalities in Hiroshima. In this study, the number of parental couples, instead of the number of children, was used as denominator for the evaluation of the abnormality frequency, since there were several instances of sibs showing the same abnormalities inherited from the carrier of either parent in both exposed and control groups. This was considered as a possible cause for biases in obtaining the true frequency of cytogenetically abnormal individuals in the human general population. The number of parental couples thus was 3,325 (or 1.35 children per couple) in the exposed, and 4,140 (or 1.19 children per couple) in the control group. Autosomal structural rearrangements dealt herewith were confined to translocation, both Robertsonian (rob) and reciprocal (rcp) types, and inversion (inv).

The frequencies of autosomal structural rearrangements of balanced type were obtained as 3.31×10^{-3} (or 11 in 3,325) for the exposed, and 2.66×10^{-3} (or 11 in 4,140) for the control, respectively. The rate of *de novo* abnormalities, or chromosomal mutations, was found to be 1 in 6 families of the abnormal probands in the exposed, and 1 in 5 in the control, giving the estimates for the mutation rate as 2.76×10^{-4} per gamete in the exposed, and 2.66×10^{-4} per gamete in the control. When the two groups were combined, the overall

rate in Hiroshima would be 2.68×10^{-4} per gamete, which is somewhat higher than the value of 1.88×10^{-4} per gamete derived from the newborn surveys by Jacobs (1981).

II-32. Reduction of Female Fertility Span after X-Irradiation at Neonatal Stages, without Teratogenic and Cytogenetic Effects on Surviving Oocytes: H. TATENO and K. MIKAMO (Dept. Biol. Sci., Asahikawa Med. Coll., Asahikawa)

In the Chinese hamster, X-irradiation at a dose of 100 rad does not induce oocyte-killing in neonates on day 0 after birth. All oocytes at this age are at pachytene stage and are highly radioresistant. On the other hand, the same treatment on day 4 causes severe oocyte-killing since a great majority of oocytes have developed to the highly radiosensitive diplotene and early dictyate stages. Consequently, only the small number of oocytes which still remain at pachytene stage can survive X-rays (Tateno and Mikamo, 1984).

Sexual maturation and fertility were examined by continuous daily vaginal smear test in females exposed to 100 rad X-rays on day 0 and 4 after birth. The number of ovarian oocytes was counted by a histological method at 5, 8 and 12 months of age. The fertility span and the oocyte number of females irradiated on day 0 were similar to those of controls, while in females irradiated on day 4 sexual maturity was delayed considerably and the estrous cycle ceased by 7 months of age evidently owing to exhaustion of ovarian oocytes. Females irradiated on day 0 were impregnated at 5, 8 and 12 months of age, but those irradiated on day 4 could be impregnated only at 5 months of age because they became sterile afterwards. On examination on day 18.5 of gestation, there was no difference between controls and both irradiated groups with respect to the numbers of corpora lutea and conceptuses and the occurrence of dead and abnormal conceptuses. Further, female pronuclear chromosomes of 1-cell zygotes were studied at 4–5 months of age. There were no differences in the incidence of structural and numerical chromosome anomalies between the irradiated groups and the controls. These results indicate that X-irradiation at pachytene stage has no teratogenic and cytogenetic effect on oocytes.

II-33. Elevated Spontaneous Mutation Rate in Werner Syndrome Cells: Kenichiro FUKUCHI, Kiyoji TANAKA and Yuichi KUMAHARA (Dept. Med. Geriat., Osaka Univ., Osaka)

Werner syndrome (WS) is an autosomal recessive disease characterized by a variety of features of premature aging. The skin fibroblast cells from patients with WS show a reduced life span *in vitro* and an impairment of DNA replication. DNA replication abnormalities have been shown to influence the degree of fidelity of the replicating unit and to provide an elevated spontaneous mutation rate in prokaryotes and in rodent cell mutants. To determine whether WS cell lines have mutator activity, we examined the spontaneous

mutation rate of WS cell lines by using the resistance to 6-thioguanine (10 μg/ml) as a genetic marker. Spontaneous mutation rates were determined by Luria and Llelbruck's fluctuation analysis. We used SV40-transformed WS cell lines, W-V and PSV811. W138VA13 and AG2804B were used as SV40-transformed normal human control cell lines. Spontaneous mutation rates were 4.7×10^{-8} and 18×10^{-8} mutations per cell per generation in W-V and PSV811, respectively. The mutation rates of WI38VA13 and AG2804B were 0.76×10^{-8} and less than 0.97×10^{-8}, respectively. These data indicate an approximately 6 to 20 fold increase in the spontaneous mutation rate in WS cell lines as compared to normal cell lines. These data suggest that WS may be a mutator mutation and that an elevated spontaneous mutation rate *in vivo* may be responsible for many features similar to the changes that occur during normal aging.

II-34. Radiosensitivity and Rejoining Rate of X-Ray-Induced DNA Single-Strand Breaks in Tuberous Sclerosis Fibroblasts: Masataka ARIMA, Yutaka YOSHIDA, Yoshihiro TAKEHANA and Harumi TANAKA (Div. Mental Retard. Birth Defect Res., Natl. Cent. Nerv., Mental Muscular Disorders, Kodaira)

Thirteen fibroblast cell strains from 11 patients with tuberous sclerosis (TS) were examined for their sensitivity to X-rays as determined from their colony-forming ability. All strains derived from normal appearing skin of patients, either sporadic or familial cases, showed sensitivity within the normal control range. Five cell lines originating from tumorous skin of 3 patients did not show hypersensitivity. It was concluded that the sensitivity to X-rays of cultured cells of TS is essentially normal. However, the mean D_0 or D_{10} values of the lines from tumorous skin tended to be lower compared to those for normal skin of patients. The formation and rejoining rate of X-ray-induced DNA single-strand breaks (SSB) were examined using the alkaline elution method. No difference was found between these cell lines in the frequency of DNA SSB directly produced by X-irradiation at any dose up to 750 rads. Kinetic analysis of the rate of rejoining of DNA SSB after X-irradiation at 500 rads, indicated that the rate of rejoining involved at least two components, an initial fast component and a slower component. TS fibroblasts were proficient as to DNA SSB repair, but they showed an increased rate of rejoining in the initial fast repair process, when compared to normal fibroblasts. It is possible that the accelerated rejoining of DNA SSB is related to a basic defect in TS.

II-35. The Loss of the Y-Chromosome in Blood Cells of Man: Takaaki ISHIHARA, Masako MINAMIHISAMATSU and Takeko ODAKA (Div. Radiat. Hazards, Natl. Inst. Radiol. Sci., Chiba)

A high incidence of a missing Y-chromosome has been known in blood cells of elderly males or patients with blood disorders such as leukemia. The mechanism of this phenomenon or the biological significance of the loss of the Y, however, has not yet been clarified. Among the radiation-exposed persons or the patients with blood diseases in which we made chromosome observations, there were a certain number of cases showing a high percentage of a missing Y in their blood cells. These cases with a missing Y have been analysed with emphasis on the following 3 points: 1) Is the missing Y specific to bone marrow cells? 2) Is the missing Y related to aging? 3) Does radiation exposure accelerate the loss of the Y? The results of the analysis may be summarized as follows: 1) Although the missing Y is not restricted to bone marrow cells, its high incidence is specific to bone marrow cells and is not recognized in peripheral lymphocytes. 2) The high incidence of the missing Y in bone marrow is related to aging, being highly observed in individuals over the age of 60. In peripheral lymphocytes, on the contrary, the incidence of the missing Y is not influenced by aging. Patients with blood disorders show a high incidence of the missing Y even in younger generations. 3) Exposure to radiation does not enhance the loss of the Y.

II-36. Immortilization of Ataxia Telangiectasia and Xeroderma Pigmentosum Fibroblasts by Transfection: Tomoko HASHIMOTO[1], Yoshiro NAKANO[2], Koji OWADA,[3] Takahiko SUKENAGA[4], Yoshihiro YAMAMOTO,[1] Noriko MATSUMOTO[1] and Jun-ichi FURUYAMA[1] ([1]Dept. Genet., [4]Dept. Radiol., Hyogo Coll. Med., Nishinomiya; [2]Dept. Oncogene, [3]Dept. Tumor Virol., Res. Inst. Microb. Dis., Osaka Univ., Suita)

Immortalization of human fibroblasts is much more difficult than that of mouse fibroblasts and human B-lymphocytes. We used a transfection technique for immortalization of human fibroblasts. Skin fibroblasts from a patient with ataxia telangiectasia (AT1OS) and xeroderma pigmentosum (XP5NI, complementation group A) were cultured in MEM with 10–15% fetal bovine serum. For transfection we used pSV40 (pBR322+simian virus 40 (SV40), full genome) and/or pMo-MSV (pBR322+Moloney murine sarcoma virus (Mo-MSV), full genome). DNA-$CaPO_4$ precipitation technique was used. Thirty to forty days after transfection, transformed foci could be observed in the cells transfected with pSV40 and pSV40+pMo-MSV. Two cell lines could be maintained for 90 population doublings, one was the AT1OS-28 (transfected with pSV40+pMo-MSV), the other was the XP5NI-1 (transfected with pSV40) cell line. These lines expressed SV40 T-antigen, the ability of

colony formation in soft agar, and hypersensitivity to γ-ray (AT1OS-28) or to UV-light (XP5NI-1). Modal chromosome number was near diploid in both cell lines and they could grow under low serum condition. Therefore, this transfection method proved to be effective to immortalize human fibroblasts.

II-37. Cloning and Characterization of a Highly Y-Specific 3.5 kb Repeated Fragment: Yutaka NAKAHORI and Yasuo NAKAGOME (Dept. Hum. Genet., Natl. Inst. Genet., Mishima)

A highly Y-specific 3.5 kb repeated fragment was cloned and characterized. Male specificity was confirmed by the standard Southern blot hybridization using DNAs derived from normal males and females. It is expected to be a very sensitive probe in the Southern blot analysis when the existance of the long arm of the Y chromosome is at issue. It was revealed that this fragment has two remarkable characteristics. First, this fragment consists of multiples of a 5 bp unit, TTCCA, and related sequences. Second, this fragment contains a somewhat unusual combination of restriction sites which is revealed in the analysis with restriction endonucleases. These findings are well interpreted by the "unequal crossover" and base substitutions of the original TTCCA unit.

II-38. Establishment of a Human Genomic Library (Partial) in an Attempt to Obtain Probes for RFLP Studies: Yasuo NAKAGOME and Yutaka NAKAHORI (Natl. Inst. Genet., Mishima)

It is attempted to establish a human genomic library from which unique DNA probes for RFLP (restriction fragment length polymorphism) studies can be obtained. Lambda phage Charon 30 was chosen as it can take a passenger DNA fragment up to 19.1 kb long into its BamHI site. DNA was isolated from leukocytes of the senior author and partially digested with a restriction enzyme, MboI. Its recognition site is ↓GATC and a fragment generated with it can be inserted into the BamHI site with a G↓GATCC sequence. After the insertion, BamHI sites may not be preserved. However, the passenger can be cut out with HpaI together with attached small pieces of lambda DNA on both ends (470 bp from the left arm and 770 bp from the right arm). It is expected that a total of 800,000 recombinant phages cover the human genome ($p > 0.99$, average fragment size 17 kb). So far, a total of 240,000 phages has been obtained which contains an amount of 3.5×10^9 bp DNA and roughly corresponds to the human genome size. Isolation of unique DNA through the Benton-Davis procedure and attempts to isolate chromosome-specific probes, through both dot-blot analysis and *in situ* hybridization, are in progress.

II-39. ヒト免疫グロブリン H 鎖定常部遺伝子の構成：コスミッドベクターをもちいたクローニング：中居純子・小平美江子・本庶 佑（阪大・医・遺伝）. **Organization of Constant Region of Human Immunoglobulin Heavy Chain: Cloning by Cosmid Vector:** S. NAKAI, M. KODAIRA and T. HONJO (Dept. Genet., Osaka Univ. Osaka)

ヒトの免疫グロブリン (Ig) の H 鎖定常部 (CH) 遺伝子は 5′-J_H-C_μ-C_δ-·····-$C_{\gamma 3}$-$C_{\gamma 1}$-$C_{\varepsilon 2}$-$C_{\alpha 1}$-···· $C_{\phi\gamma}$-···-$C_{\gamma 2}$-$C_{\gamma 4}$-$C_{\varepsilon 1}$-$C_{\alpha 2-3}$′ の順に配列しており，マウスとは違って C_γ·C_ε·C_α の遺伝子セットが重複した構造であることが明らかになっている．またこの領域には，制限酵素切断部位多型のみならず数十キロ塩基対 (kb) 以上にわたる欠失の例が知られている．

われわれは，ヒト IgCH 遺伝子セットの重複単位の長さとその間の距離や構造を知るために，この領域の全構成の解明を目的として以下の実験を行った．ヒト末梢血より得た DNA を Taq I で部分分解し，pJB8 をベクターとしてコスミッドライブラリーをつくり，すでにわれわれの研究室で単離した各 CH 遺伝子の DNA 断片をプローブとして多数のクローンを分離した．得られたクローンは，(a) 5′-J_H-C_μ-C_δ を含む C_δ から 3′ へ 10 kb の領域，(b) $C_{\gamma 3}$ の 5′ 側 25 kb から $C_{\alpha 1}$ までの領域，(c) $C_{\gamma 2}$ から $C_{\varepsilon 1}$ を含む約 50 kb であった．C_δ から $C_{\gamma 3}$ までは 35 kb 以上はなれていることがわかったが，まだ各領域間を連結するクローンは得られていない．末梢血ライブラリーより分離したクローンには $C_{\gamma 3}$～$C_{\gamma 1}$ 域のものが非常に多いが，C_α から 3′ 側のものは得られていない．ライブラリーに偏りがあるのではないかと考えられる．また，J_H の 5′ 側で遺伝子再構成を起こしていると考えられるクローンが得られた．

II-40. Cloning of Human β-Globin Gene Cluster: Atsuo MORI and Zen-ichi OGITA (Dept. Pathol. Biochem., Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama)

Several techniques have been published for the isolation of plasmid DNA. By a combination of few simple and effective techniques and high pressure liquid chromatography system we established a new inexpensive and effective method. We applied this method to cloning of human β-globin gene cluster. The human β-globin gene cluster was combined with cosmid vector pJB8 at a BamHI site. The recombinant DNA was packed in lambda phage and transduced to *E. coli* HB101. *E. coli* was grown selectively on LB medium supplemented with ampiciline and amplified by the addition of chloramphenicol. Then the recombinant DNAs were collected by our method. It was found that the human β-globin gene was cloned effectively.

II-41. Evaluation of the Embryo-Transfer through Cervical Canal Using Mice: Yoshiyasu HOMBO, Shosuke TAKAMURA (Dept. Obst., Kanazawa Natl. Hosp., Kanazawa) **and Zen-ichi OGITA** (Dept. Pathol. Biochem., Oriental Med. Inst., Toyama Med. Pharm. Univ., Toyama)

The procedure for the *in vitro* fertilization and embryo-transfer seems to have been gradually improved, but there are still problems in it. One of the serious problems to be solved is the very low success rate of the implantation in human, 3–14% per a transferred embryo. In the case of mice, the success rate of the embyo-transfer and implantation is high, 70–80% per a transferred embryo. In mice, the embryo-transfer into the uterine cavity is performed through the uterine wall, surgically. In human, it is done through the cervical canal, non-surgically. We think that the big gap in the success rates between the two species is partially due to the difference in the embryo-transfer methods. So we did the embryo-transfer both surgically and non-surgically using mice. We chose black mice as the donor of the *in vivo* fertilized embryo and white mice as recipients of these embryo. The results are the following.

	Pregnancy rate	Delivary rate per a transferred embryo
Surgical transfer	3/5 (60%)	11/88 (13%)
Non-surgical transfer	3/18 (18%)	7/96 (7%)

As we had expected, the transfer through the cervical canal is inferior to the surgical one, in both pregnancy and delivary rate. But in our experiment, the success rates is still low. So it is important for us to improve our instrument and sharpen our skill in order to get better rates and confirm the evaluation above.

II-42. Selection of Human B Cell Hybridoma by the Use of Monoclonal Anti HLA Class II Antibody and Fluorescence Activated Cell Sorter (FACS): Yasuharu NISHIMURA, Fumiki HARADA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

We have established the selection method of human B cell hybridoma by checking the polymorphic marker of HLA class II molecules which are selectively expressed on human B cells and monocytes. For this purpose, we established and collected three HLA-DR non-identical human B cell lines and ten monoclonal antibodies (MoAbs) to the polymorphic determinant of HLA class II molecules. GM-1500 is a hypoxanthine guanine phosphoribosyl transferase (HGPRT) deficient clone of human myeloma cell line and this cell line is negative for HLA-DR2. Healthy donor YN is a heterozygote of HLA-DR1/DR2. Peripheral blood lymphocytes of a donor YN were fused with GM-1500 using poly-

ethylene glycol. Cells were stained with MoAb HU-30 against HLA-DR2 by the indirect immuno-fluorescence staining. Cells were then sorted by FACS to collect HLA-DR2 positive large human B cell hybridomas. These hybridomas were cultured in normal medium without HAT and cloned by the limitting dilution method. Since GM-1500 secreted IgG_1 (γ_1/κ), we checked the heavy and light chains of human immunoglobulin other than γ_1 and κ chains in the culture supernate of hybridomas to confirm the establishment of human B cell hybridomas. The expression of HLA-class II molecules reactive with MoAb 17.15A which were expressed on GM-1500 myeloma cell but not on the B cell from a donor YN was also investigated to exclude the possibility that cells secreting immunoglobulins might be spontaneously transformed B cells from a donor YN. By this selection method B cell hybridomas were concentrated about 25,000 times from the bulk cells after cell fusion. The probability of the establishment of human B cell hybridoma increased about ten times as compared with classical HAT selection method.

III-1. Heteromorphisms of Ag-Stained Nucleolar Organizer Regions in Human D and G Chromosomes: **Yoshiaki KODAMA and Akio A. AWA** (RERF, Hiroshima)

It is known that the nucleolar organizer regions (NORs) are located on the short arm of D and G chromosomes of man. Recently, new techniques developed for the differential staining of the NORs have offered an opportunity to study these specialized regions of chromosomes. In the present study, 172 children (86 males and 86 females) of atomic-bomb survivors were studied for the variation of their NORs. Chromosome preparations were made from whole blood cultures, and 10 cells stained by the combination of silver staining method and G-banding method were analyzed in each sample. The approximate size of the Ag-stained NORs was scored on a graded scale of 0–3. Our investigation revealed that the Ag-stained chromosomes did not occur randomly, and the inter-chromosomal distribution of the Ag-stained deposit showed a consistent pattern for each individual. A mean Ag-stained NORs number of 8.6 chromosomes was obtained with a mode of 9. No difference was found between females and males. Chromosomes 13 and 21 showed high staining intensity of the NORs, while chromosome 22 showed low intensity. The differences in the size of Ag-stained NORs were examined for each of homologous pairs. About 35% of the pairs of chromosomes 13, 14, 15 and 22, and 23% of the pairs of chromosome 21 were found to have different but distinct Ag-stained NORs in size. About 85% of the present sample cases showed variations at least in one of the homologous pairs. These chromosome markers appear to be useful for characterization of each individual as well as for distinction of each homologous chromosomes.

III-2. Studies on Characterization and Staining Affinities of Acridine Derivatives to Human Chromosomes: **Kouichi MAMBA, Noriaki SAKATA, Mutsuo KITAHAMA** (Dept. Legal Med., St. Marianna Univ. Sch. Med., Kawasaki) **and Akira UCHIUMI** (Natl. Chem. Lab. Indust., Tsukuba)

The present study was carried out to examine the characterization and staining affinities of seven acridine derivatives as new fluorescent dyes to human chromosome samples. The seven acridine derivatives synthesized were 9-aminoacridine, diacridinocyanul, monoacridinocyanul, 2-ethoxy-6,9-diaminoacridine lactate, 3,6-diaminoacridine hydrochloride, 9-acridylanthrahydrazone and di-9-acridylphenylhydrazone. The following results were obtained. 1) The colors of fluorescences of 9-aminoacridine, diacridinocyanul and monoacridinocyanul showed pale blue, the absorption maximum values were between 401 and 424 nm and the dissociation constants (pK_a) were between 9.0 and 10.1. 2) The colors of fluorescences of 3,6-diaminoacridine hydrochloride and 9-acridylanthrahydrazone showed orange, the absorption maximum values were between 440 and 456 nm and pK_a is between 5 and 6. 3) The colors of fluorescences of 2-ethoxy-6,9-diaminoacridine lactate and di-9-

acridylphenylhydrazone showed yellow or yellowish-green, the absorption maximum values were between 413 and 462 nm and pK_a is between 5 and 6. 4) 3,6-Diaminoacridine hydrochloride showed less staining affinity among those derivatives to the samples. 5) Double staining with di-9-acridylphenylhydrazone/methylgreen produces a weak banding pattern near the centromere position of a pair of C group chromosomes.

III-3. Staining Pattern of Nucleolus Organizer Regions in Man: Masahiro ITOH (RERF, Nagasaki)

Nucleolus organizer regions (NORs) are present in the short arms of human chromosomes of group D and G, and specific staining of NORs using the N-banding technique is available. The number and size of NORs to be stained vary with the individual, and its variation is recognized as a serial pattern. It has also been reported that NORs variance differs with different races and ages. However, the NOR staining pattern in Japanese has not been clarified adequately since reports are scarce and the number of subjects is restricted. NOR staining was performed on 221 individuals ranging in age from 13 to 74 yrs., who were subjected to chromosome study at RERF Nagasaki Branch. They were divided into three age groups, *i.e.*, 106 persons in young (13–17 yrs.), 55 in middle (40–59 yrs.) and 60 in old (60 yrs. or more) groups. These groups were compared in terms of the number and size of NORs that were recognized in the D and G group chromosomes. The average number of NORs was 7.70, 7.55 and 7.55 for young, middle and old age groups, respectively. D-NORs numbered 4.67, 4.44 and 4.50, and G-NORs 3.03, 3.11 and 3.05 for respective age groups. The average number of D-NORs and G-NORs in all individuals examined was 4.57 and 3.05, respectively, totalling 7.62. This study is still ongoing and hence statistical analysis has not been performed. So far, however, there has been observed no decrease in number of NORs with age and no difference between the sexes.

III-4. 各種培養条件下における染色体脆弱部位の出現頻度：杉尾嘉嗣・梶井　正（山口大・医・小児）. **Frequencies and Distribution of Common Fragile Sites in PB Lymphocyte Cultured in Folate-Free and BrdU-Added Media:** Yoshitsugu SUGIO and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

7～31 歳の正常男女 8 人と同年齢の fra(x) 陽性男女 4 人の末梢血リンパ球を次に述べる 4 種の培養条件下で 4 日間培養し，染色体標本を作製した．1) Eagle の MEM（葉酸を 1 mg/*l* 含有），2) 葉酸を除いた MEM（MEM-FA と略記），3) MEM-FA で培養最後の 24 時間前に pH を 7.6 に上げたもの（MEM-FA, pH↑ と略記），4) MEM に BrdU 20 μg/ml を培養最後の 4～6 時間加えたもの（MEM＋BrdU と略記），の 4 種で，いずれも PHA と仔ウシ血清 5% を加えた．通常の

Giemsa 染色標本について各培養ごとに 200 の分裂像を観察し，染色体上のギャップと切断の部位を記録した後に脱色，G-分染し，ギャップと切断の部位を同定した．

正常者と fra(x) 陽性者では，fra(x) 以外に差はなかったので一括する．MEM でギャップと切断の平均値は 7.0%, MEM-FA で 36.2%, MEM-FA, pH↑ で 43.2%, MEM+BrdU で 19.0% だった．そのうち 1% 以上の頻度で認めた部位は，MEM で 3p14, MEM-FA と MEM-FA, pH↑ で 3p14, 6q26, 16q23, 1p31, 1q44, 3q26, MEM-BrdU で 3p14, 5p14, 9p13 であった．このうち common fragile sites として報告されていない新しい部位は，3q26, 5p14, 9p13 の 3 個所であった．cancer breakpoints と一致するのは 1p31 (neuroblastoma; 1p31–1p36) と 3p14 (small cell lung cancer; 3p14–3p23) で，oncogenes と一致するのは 1p31 (N-ras; 1p22–1p31) であった．Fra(x) 陽性者では，MEM-FA, pH↑ における fra(x) の出現率が MEM-FA に比べ有意に高かった．

III-5. Preliminary Cytogenetic Study on the Methotrexate-Induced Fragile Sites in a Mentally Retarded Population: Tadao ARINAMI,[1] Ikuko KONDO[2] and Susumu NAKAJIMA[1] ([1]Ibaraki Pref. Colony, [2]Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

To elucidate the association of fragile sites including Fra(X)(q27) with mental retardation, the distribution of fragile sites in methotrexate-treated cells was analyzed in 50 mentally retarded males. Chromosomal analysis was performed on peripheral blood lymphocytes which had been cultured in TC199 medium for 68 hr and further cultured in the presence of methotrexate (0.01 mg/ml) for 24 hr. Fifty Giemsa-stained metaphases per individual were analyzed and fragile sites were confirmed by GTG method. A total of 199 fragile sites were found at 43 bands on 17 chromosomes in the 2,570 metaphases (mean frequency 4.7%). The fragile sites observed here were identical with the 44 known ones (Sutherand and Hecht, 1984). The frequency of cells with fragile sites ranged from 0% to 6% in most of the individuals. However, 16% to 26% of cells had fragile chromosomes in four individulas and the distribution of fragile sites was nonrandom in all the four. In three out of the four individuals, the fragile sites were located at 3p14 and 16q23. In addition, Fra(X)(q27) was found in 7% of examined cells from one male with severe mental retardation. These data suggest that common fragile sites such as 3p14 and 16q23 are associated with mental retardation in a small group of individuals in the mentally retarded population. In addition, about 2% of the mentally retarded males may have Fra(X)(q27) syndrome in Japan.

III-6. 脆弱 X 症候群 2 家系の報告と細胞遺伝学的検索：池田琢哉・宮城仲健・平山清武（琉球大・医・小児）. **Fragile X Syndrome. Report of 2 Families and Cytogenetic Studies:** Takuya IKEDA, Chuken MIYAGI and Kiyotake HIRAYAMA (Dept. Pediatr., Ryukyu Univ. Sch. Med., Okinawa)

脆弱 X 症候群は，葉酸を除去した条件下で細胞培養したときの X 染色体の脆弱性を特徴とする X 連鎖性劣性遺伝性の精神薄弱症候群である．その頻度は，欧米では男性 1,000 人に 1 人といわれているが，Son Blomquist らによると，その頻度は高すぎ，実際には新生児 3,000 人に 1 人ぐらいであろうと推測されている．本症候群の症状は，精神遅滞を除けば共通する症状に乏しい．精神遅滞の程度は中等度ないし重度の例が多い．また，患者の多くは会話の障害を伴う．多動やかんしゃくをおこしやすい．自閉的傾向などの行動異常が認められることがある．本症候群の小児期における外見上の特徴は乏しいが，大きく単調な耳介，面長の顔，眼窩上縁の突出などの顔貌と思春期以降の巨睾丸が認められる．私たちの症例では，精神遅滞のほかに，面長の顔，大きく単調な耳介が 3 症例に一致してみられた．ほかに，症例 1 では，反響言語と多動を認め対人接触障害が著明であった．症例 2 では，頭囲の拡大と，言語には軽度の構音障害があり全体的に滑らかさを欠いていた．症例 3 では，年齢に比し睾丸が拡大していた．母親は，軽い精神薄弱で彼女からも脆弱 X 染色体が検出された．

III-7. Sister Chromatid Exchanges: Sex Difference in Base-Line SCE Frequency: Mimako NAKANO, Junso NARUTO and Akio A. AWA (Dept. Clin. Lab., RERF., Hiroshima)

In our previous observations there was a slight but positive correlation of SCE frequencies with increasing age in males, but not in females as the range of age between 10 and 79. SCE value in males requires careful considerations, since age-dependent difference in SCE frequencies may reflect chronic exposure of individuals to potential SCE-inducing environmental mutagens, such as smoking. Therefore, it is relevant to investigate young individuals, who are considered as free of smoking or alcohol intake, to obtain sex difference affecting the frequency of base-line SCEs. The present study was undertaken on 78 children of both sexes at the age of 10–19 years born to A-bomb survivors (F_1). SCE frequencies from the two sexes were carefully investigated for the reproducibility of the results. All of our preliminary data derived from three individual studies showed that mean SCE frequencies were lower in males than in females. The differences between two sexes for each study were 0.75, 1.02 and 0.42 per cell, respectively with an average of 0.70 per cell. The numbers of SCEs per X and Y chromosome were 0.203 and 0.035, respectively, the difference being 0.17 between the two sex chromosomes. In regard to autosomes, there was a tendency that slightly higher frequencies of SCEs were observed in females than in males in all chromosomes or chromosome groups. In the two of three studies, large differences in the SCE frequency per chromosome between the males and females was noted in the no. 2 chromosome. It remains unclear whether or not the elevated SCE frequency in the no. 2 chromosome are the contributing factors affecting sex difference in the base-line SCE frequency.

III-8. Mechanism of Highly Increased Sister Chromatid Exchange (SCE) in Bromodeoxyuridine (BrdU)-Substituted Bloom Syndrome Cells: Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Nankoku)

The most prominent cytogenetic characteristic of Bloom syndrome is an increased rate of SCEs in cells labeled with BrdU for 2 cell cycles. The data from endomitotic analysis and three-way differentiation reveal the cell cycle timing of the occurrence of BS SCEs. The analysis of single and twin SCEs in BrdU-labeled endomitosis demonstrated that although an equal number of SCEs occurred in each of the 2 cell cycles in normal cells, most of BS SCEs appeared during the second cell cycle as single SCEs ($SCE_1=4.9$, $SCE_2=70$). Three-way differentiation analysis indicates that BS cells are highly sensitive to BrdU and that BS cells which incorporated BrdU heavily during the first cell cycle can not proceed to the third cell cycle. In BS cells which proceed to third mitosis in the presence of BrdU, the exact frequency of SCE_1 was rare (3.28 ± 0.13). In contrast, high frequencies of SCE_2 (63.32 ± 2.13) and SCE_3 (73.08 ± 2.15) were observed in three-way differentiated BS cells. These findings were strongly supported by recent finding of Chinese hamster mutant line EM9 with high (80–100) baseline SCE; the majority of the high baseline SCE appear to be a consequence of BrdU incorporation, and they arise during replication of DNA containing BrdU in a template strand. The alkaline elution patterns of DNA newly replicated on a BrdU-containing template are significantly altered compared with those seen on an unsubstituted template. These findings strongly indicate that highly increased baseline SCE levels observed in BrdU labeled BS and EM9 cells are caused by replication of DNA containing BrdU in a template strand.

III-9. Analysis of DNA Chain Growth Rate in Bloom's B-Lymphoblastoid Cell Lines (Normal and High SCE) by DNA Fiber Autoradiography: Takahiro TAGUCHI and Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Nankoku)

Bloom syndrome (BS) is an autosomal recessive disorder characterized cytogenetically by a greatly increased level of sister chromatid exchange (SCE) in cells labeled with bromodeoxyuridine (BrdU). Two conflicting results have been reported on DNA chain growth rate in BS cells. Hand and German (1975 and 1977) reported a retarded rate of replication fork movement in BS skin fibroblasts and PHA stimulated lymphocytes, while Ockey *et al.* (1979) pointed out normal rate of DNA chain growth in BS skin fibroblasts using DNA fiber autoradiography. We have undertaken to clarify the exact property of DNA replication fork movement in four BS B-lymphoblastoid cell lines (two with normal SCE; BS1-1, BS2-1 and the others with high SCE; BS1-2, BS2-2). Two types of protocols were done: one with pulse labeling (^{3}H-thymidine; 30, 60 and 120 min) and the other with hot pulse-warm chase (^{3}H-thymidine; 20+40 min). Chain growth rates were 0.72(BS1-1), 0.76(BS1-

2), 0.77(BS2-1) and 0.77(BS2-2) μm/min on the average. Replicon sizes were ranging from 20 to 130 μm in all cell lines. These findings strongly indicate that there is no difference of DNA chain growth rate in BS B-lymphoblastoid cells regardless of normal and high SCE rate and that high SCE character is independent of DNA chain growth. Considering the recent report (Shiraishi, 1983) that most of BS SCEs were caused by BrdU and spontaneous SCE level is almost normal level in BS cells, the present finding of no difference in DNA chain growth in high SCE BS cells with normal SCE is reasonable.

III-10. Induced Sister Chromatid Exchanges in Fibroblasts from Patients with Bloom's Syndrome: Takayuki KURIHARA and Masao INOUE (Cent. Lab., Kanazawa Med. Univ., Uchinada, Ishikawa)

Induced sister chromatid exchanges (SCEs) in Bloom's syndromes (BS) cells have been investigated as a function of survival measured by colony formation after ultraviolet (UV), methyl-nitrosourea (MNU) or ethyl-nitrosourea (ENU) treatment. In normal cells, these three mutagens induced the same frequency of SCEs at doses yielding equal levels of survival. However, in BS cells, SCEs induced by UV, MNU or ENU were about a 2-fold, 6-fold or 30-fold higher than that in normal cells, respectively. These results suggest that the mechanism of SCE induction in BS cells is susceptible to the mutagens and that ENU is more effective in SCE induction than the other mutagens.

III-11. Mutagenicity of Immunosuppressants: I. Evaluation of *in vitro* Sister Chromatid Exchange Induction by Cyclosporin A: Kenji YUZAWA,[1] Katashi FUKAO,[1] Yoji IWASAKI,[1] Ikuko KONDO[2] and Hideo HAMAGUCHI[2] ([1]Dept. Surg., [2]Dept. Hum. Genet., Univ. Tsukuba, Ibaraki)

Recently, Cyclosporin A (CyA) has been extensively used as an effective immunosuppressant in the organ transplantation in U.S.A. and Europe. Although it has been reported that CyA does not have mutagenicity nor carcinogenicity, human cells have not been used in these studies. To examine whether CyA has mutagenic potentials against human cells, we analyzed sister chromatid exchange (SCE) induction by CyA using human lymphocytes *in vitro*. Heparinized peripheral blood lymphocytes from three healthy volunteers were cultured in the presence of CyA for 2 hr. Then, PHA-P and BrdU were added to the culture medium and the cells were further cultured in the dark for 4 days. Lymphocytes were harvested in the usual way. After FPG staining, frequencies of SCE were counted. Both blastoid index and mitotic index decreased in the lymphocytes treated with CyA, depending on the CyA concentration. SCE frequencies increased slightly in the lymphocytes treated with 1 μg/ml CyA in all the three individuals and the difference was statistically significant.

This result indicates that CyA has an SCE inducibility. The finding also suggests that CyA has a mutagenic effect on human lymphocytes.

III-12. Accumulation and Persistence of Cyclophosphamide-Induced Sister Chromatid Exchange in Murine Peripheral Blood Lymphocytes: Tatsuya TAKESHITA, Makoto HIGURASHI (Dept. Health Sci., Yamanashi Med. Coll., Yamanashi) **and M.K. CONNER** (Dept. IEHS, GSPH, Univ. Pittsburgh, USA)

SCE frequencies were evaluated in peripheral blood B-cell lymphocytes cultured at various times following treatment of BDF_1 mice with 3.0 mg/kg cyclophosphamide (CP). In blood samples drawn at 5 min, 20 min, 35 min, 1 hr, 3 hr, and 24 hr after a single injection of CP, the respective mean SCE frequencies (3 mice/group) were 17.1 ± 2.0, 19.9 ± 3.0, 19.3 ± 1.8, 21.6 ± 2.4, 20.6 ± 2.3, and 16.0 ± 2.4, respectively. At 3 days post-exposure, SCE frequencies (12.9 ± 1.4) were near baseline levels (10.9 ± 1.2). The rapid formation of SCE-inducing lesions in lymphocytes reflects the facile metabolism of CP. At 1 hr and 24 hr following the last of 2, 4, or 6 multiple injections (3 mg/kg; every other day), SCE responses accumulated with increasing number of injections. Accumulation of SCEs was also observed at various times (24 hr, 24.8 ± 1.5; 3 d, 17.6 ± 1.4; 1 wk, 16.9 ± 1.3) after 12 serial injections (3 times weekly) and increased SCEs (13.4 ± 1.1) were still apparent at 4 wk post-exposure.

III-13. A Chromosome Study of the Congenital Defectives and Their Relatives: Tetsuji KADOTANI and Yoko WATANABE (Kadotani Med. Res. Found., Higashi-hiroshima)

During a peiod from November 1975 to the end of July 1984, 156 cases were collected for chromosomal studies. The chromosome studies were performed with the standard blood cultures. The chromosomes were analysed following the conventional Giemsa, the G- and Q-banding procedures. Seventy one out of the 156 cases were congenital defectives and the remaining 85 cases were their relatives. Forty two out of the 71 cases of congenital defects were found to possess normal karyotypes, leaving 29 cases showing abnormal karyotypes. Among the abnormalities, 7 cases were found to be transmitted through the parental line, while the remaining 22 cases seemed to be of sporadic origin, since the parents of those cases were chromosomally normal. Out of the 29 cases with chromosome abnormalities, 27 cases showed autosomal abnormalities, while the remaining 2 cases had sex-chromosome abnormalities. Then it is apparent that the chromosome study serves as an important tool for the clinical diagnosis of the defectives and their relatives seeking genetic advices.

III-14. Studies of Chromosome Abnormalities in Myotonic Dystrophy: N. NATORI, G. SATO, T. KONOSU and K. NAKAGAWARA (Natl. Nishitaga Sanat., Sendai)

Studies of chromosome abnormalities in 15 patients with myotonic dystrophy were performed. Peripheral blood lymphocytes were cultured in folic acid-deficient culture medium (MEM-FA) for 3 days, and aminopterin was added 24 hr before harvest. A total of 100 metaphases were examined looking for fragile sites, and the frequency of fragile sites in 2% or more of the metaphases was judged as positive. Parental chromosomes were not studied. Results obtained were as follows: 1) Structural abnormalities were not detected by G-banding. 2) 4 out of 15 patients had fragile sites, and by the addition of aminopterin 4 other patients with fragile sites were found. 3) The most frequent fragile site was seen at 6q12 or 13. 4) Younger patients had more fragile sites than elders, though further accumulation of cases is needed. We considered that many patients and parents with myotonic dystrophy should be studied for fragile sites.

III-15. Chromosome Abnormalities in Mentally Retarded Persons with Convulsive Seizures: Atsushi IESHIMA, Taeko YORITA and Kenzo TAKESHITA (Div. Child Neurol., Tottori Univ. Sch. Med., Yonago)

We investigated the frequencies of chromosome abnormalities in mentally retarded patients with convulsive seizures. 19 patients with chromosome abnormalities were found in this study (14%). 10 cases of 71 patients with tonic clonic convulsion had chromosome abnormalities, including mosaic 22 trisomy, 4q trisomy, 1q monosomy, 21p+, r(17), r(20), r(14) (2 cases), 4q+, and Klinefelter syndrome (48,XXXY). Five of 11 patients with neonatal convulsions showed chromosome abnormalities, including 18 trisomy (3 cases), isochromosome 18, and 15q trisomy. Three of 19 patients with West syndrome were Down syndrome (2 cases) and r(18) syndrome, respectively. Two of 7 patients with Lennox syndrome were r(14) and mosaic r(20). One of 11 cases with atypical absence seizures had an inversion 10. No case of chromosome abnormalities was observed in 14 patients with partial seizures. Among the above 19 patients, 7 patients were diagnosed as having chromosome abnormalities after suffering from convulsions. The case with 46,XX,del(1)(q42) had multiple congenital anomalies, but other 6 cases, including two cases with r(14), each one case of mosaic r(20), r(17), 22 trisomy mosaicism and inversion 10, had a few dysmorphic features, but no congenital anomalies.

III-16. Very Rare Heteromorphic Chromosomes 1 and 10 as Revealed by C-Banding Method: **Naoki NOMOTO, Yuri MIYANOMAE, Akira YOSHIDA** (Dept. Pediatr., Kyoto City Child Welfare Cent., Kyoto) **and Osamu NAGAUCHI** (Dept. Clin. Lab., Kyoto City Hosp., Kyoto)

It is well-known that the heteromorphism of C-bands in human chromosomes 1, 9, 16 and Y can be readily demonstrated by using the C-staining method. Recent studies have revealed that the C-band heteromorphisms are detected in the other chromosomes as well, including Nos. 4, 5, 6, 12, 14 and 15. We report here two families with very rare heteromorphic chromosomes demonstrated by the C-staining method. In one family, an extremely large C-band was found on a chromosome 1, the size of which was designated as CBG50 according to ISCN (1978). This chromosome was found in the proband's mother and sister. The proband and his elder brother had a heteromorphic No. 1 with a relatively large C-band (CBG40), which was transmitted from their father. The other family showed a chromosome 10 with a distinct C-band, which was found in the proband's father and elder sister. To our knowledge, the heteromorphic No. 10 has not previously been reported.

III-17. A Case of *de novo* Intertitial 3q Deletion: **Noriko OKADA, Makiko OHSAWA, Yukio FUKUYAMA** (Dept. Pediatr. Tokyo Women's Med. Coll., Tokyo) **and Tomoko HASEGAWA** (Div. Genet., Clin. Res. Inst., Natl. Med. Cent., Tokyo)

A female patient with a deletion of 3q12-q21 is presented. The patient was delivered after an uneventful 40-week and 3-day gestation. Her parents were healthy and unrelated. There was no family history of congenital anomaly or developmental delay. The birth weight was 2,600 g, body length 47.2 cm, head circumference 33.0 cm and chest circumference 30.5 cm. Fetal distress and mild asphyxia were present at birth. The patient had peculiar features including plagiocephaly, torticollis, hypertelorism, epicanthus fold and high arched palate. Other abnormalities included progressive S-shaped scoliosis, multiple joint contracture, multiple skin pigmentation and renal anomaly involving incomplete duplication of the collecting system on the right side. Psychomotor development was delayed with a DQ of 16. From laboratory findings, iron deficiency anemia and excess serum IgG were recognized. Cytogenetic analysis was performed using a peripheral blood culture. The karyotype of the patient was 46,XX,del(3)(q12q21). Her parents showed a normal karyotype. At 8 years of age, the patient could walk only 5 to 6 m unaided without a corset, and was unable to speak any meaningful words. Growth retardation was also observed.

III-18. A Case of Ring Chromosome 9: Noriyoshi KASA (Dept. Pediatr., Natl. Okayama Hosp., Okayama) **and Ryozo KASAI** (Asahigawa Jidoin Child. Hosp., Okayama)

The patient was a two days old male newborn who was the second child of unrelated and healthy parents. Maternal age was 25 years and paternal 30. His only sibling was a one year old healthy girl. He was born after 39 weeks normal gestation, weighing 2,320 g. He was admitted because of ambiguous genitalia and odd face. The following abnormalities were observed; microcephaly, trigonocephaly, ocular hypertelorism, mild exophthalmos, antiverted nostrils, long philtrum, small mouth, malformed ears, short neck, short and broad fingers, clynodactyly of the 5th fingers, hypospadia, micropenis, undescended testes, hypertrichosis of back, shoulder and hip, heart murmur and short stature. These clinical features were similar to those of previously reported cases of ring chromosome 9. The high resolution banding studies in peripheral leucocytes revealed that most of the cells (82.3%) had a karyotype of 46,XY,r(9)(p24.1q34.3) and the remainder included the cells of 45,XY,−r(9), 46,XY,dicr(9) or others. Both parents had normal karyotypes. Erythrocyte AK1 and GALT activities, measured by the method of Beutler, were 190 IU/gHb (control; 215.3±23.8) and 22.4 IU/gHb (control; 21.6±2.9), respectively. Therefore it was suggested that the AK1 locus can be excluded from the most distal region within the band 9q34, to which the locus was formerly assigned (HGM 7, 1983).

III-19. Regional Mapping of Human Chromosome 9: Exclusion of Genes for Adenylate Kinase 1 (AK1) and Galactose-1-Phosphate Uridyltransferase (GALT) from 9p22→qter: Hidetsune OISHI (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai), **Itsuro NISHIGAKI** (Dept. Intern. Med., Kyoto Pref. Univ. Med., Kyoto) **and Tsutomu YAMANAKA** (Cent. Hosp., Aichi Pref. Colony, Kasugai)

Previous studies have mapped the GALT [EC 2.7.7.12] structural gene locus to chromosome 9, either at band 9p13 or band 9p22. The SRO for AK1 [EC 2.7.4.3] locus is also confirmed to be band 9q34. In the present study, we examined the activities of AK1 and GALT in a patient with partial monosomy of chromosome 9. The patient was born on April 6, 1977, after 42 weeks of gestation, as the second child of a 27-year-old mother and a 30-year-old father. The parents were not consanguineous. At birth, the boy weighed 3,950 g and was found to have multiple abnormalities: mental retardation, trigonocephaly, upslanting palpebral fissures, anteverted nostrils, low-set and deformed ears, long philtrum, high-arched palate, wide-set nipples, hernias and syndactyly of toes. Chromosomal analyses of the patient revealed that one of the chromosomes of group C was lost. By the differential staining with trypsin-Giemsa (G) and BrdU-acridine orange (R) methods, it was confirmed that a part of the short arm of chromosome 9 was deleted. However, no

evidence of translocation of the deleted material onto other chromosomes was noted. Therefore, the karyotype of the patient could be written as 46,XY,del(9)(p22). His parents had apparently normal chromosome sets. The activities of AK1 and GALT were measured in erythrocytes of the patient and his parents. The levels of these enzymes in all members were within normal limit. Therefore, the gene loci for AK1 and GALT could be excluded from region 9p22→pter.

III-20. A Case Report of a *de novo* X-10 Reciprocal Translocation: Junko TACHIKURA, Kenji NARITOMI and Tamotsu TERAWAKI (Dept. Pediatr., Kagoshima Univ., Kagoshima)

A girl with an X-10 translocation was reported, who was observed during a period of 2 days to 15 months after the birth. The proband, the third child of healthy non-consanguineous parents, was born at 41 weeks after uneventful pregnancy; birth weight 2,280 g. She was suffered from asphyxia (Apgar 2 points) and recovered by O_2 therapy at that time. Physical examination revealed hypotonia, closed fontanelle, left microphthalmia, low set ears, micrognathia, retrognathia, widely spaced nipples, proximally set thumb, *etc.* She had few voluntary movements and no neonatal reflexes, but had frequent generalized chronic convulsions. EEG revealed suppression-burst. The gradual enlargement of venticles was found in brain CT scanning. Chromosomal study using high resolution banding technique revealed a balanced reciprocal translocation, 46,X,t(X;10)(p11.3;p13). Late replication in a normal X chromosome was confirmed in all the 20 cells examined, by using BrdU-AO method. Karyotypes of the parents were normal. She was fed with a nasal tube because of the inability of the swallowing. Height and head circumference are little increased inspite of good weight gain. Psychomotor development was severely retarded. She died of pneumonia at 15 months old. Autopsy revealed the brain hypoplasia and left persistent hyperplastic primary vitreous.

III-21. A Case of Partial Duplication 11p Syndrome: Hirofumi NAKAJIMA,[1] Seiichi FUKUDA,[1] Miwako TSUNOSUE,[1] Takeo HASHIMOTO,[1] Masato MARUYAMA,[2] Kyoko NISHIJIMA[2] ([1]Dept. Neomat., [2]Lab., St. Mary's Hosp., Kurume) **and Yasuo NAKAGOME** (Natl. Inst. Genet., Mishima)

Anomalies of the short arm of No. 11 chromosome are extremely rare, especially in Japan. The patient, 4 years old female, was born on May 16, 1980 by vertex presentation, and vacuum extraction was used. Her birth weight was 2,570 g in a 41 weeks gestation. Apgar scores were 6 at one minute and 9 at five minutes. She is the first child of normal parents. There is no consanguinity. There is no family history of congenital malformation and genetic problems. The maternal and paternal ages at the time of her birth were

23 and 28 years, respectively. She was referred to St. Mary's Hospital Neonatal Center because of fever and stridor at the age of 4 days. She showed cephalohaematoma, the abnormality of eye movement, hypertrichosis and a prominent forehead. The computed tomography of her brain showed slightly subdural haematoma. She was put on to conservative treatment and discharged well at the age of 34 days. At the age of 67 days she was readmitted to our medical center because of feeding difficulties, poor weight gain and worsening of retraction. She was discharged again at the age of 7 months. She requires tube feeding because of weak sucking. She has severe phychomotor retardation and convulsions. Intractable epilepsy has been confirmed by EEG. The computed tomography of her brain was checked again and showed brain atrophy. She has had recurrent upper respiratory tract infection and pneumonia. Other abnormalities include prominent forehead, flat nasal bridge, downslanting palpebral fissures, hypertelorism, strabismus, hypertrichosis, muscle hypotonia, and marked funnel chest. Dermatoglyphic study showed a predominance of ulnar loop patterns of the finger and palmar axial triradii in the t'' position bilaterally. Chromosome analysis with G banding revealed 46,XX,inv dup(11)(p15.5 →p13). The karyotypes of her parents are normal.

III-22. Relationship between Coagulation Factors VII and X, and Chromosome 13: **Yoshimitsu FUKUSHIMA, Marie NISHIHARA, Yoshikazu KUROKI** (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama) **and Atsuo IIZUKA** (Div. Hematol., KCMC, Yokohama)

It is proposed that the structure genes of coagulation factors VII and X (VII-X) are located on the long arm of chromosome 13 (Pfeiffer *et al.*, 1982; de Grouchy *et al.*, 1984). We analyzed activity and antigen of VII-X in 5 patients with abnormal chromosome 13. Case 1 through 3 had a karyotype with 46,XX,del(13)(q32). Case 4 was 13 trisomy syndrome (47,XX,+13). Case 5 was suffered from retinoblastoma and the karyotype was 46,X,t(X;13)(p11.21;q12.3). Measurements of activity (VII-C) and antigen (VII-A) of coagulation factors VII and those of X (X-C, X-A) were performed in these 5 cases. Results were 58% for VII-C, 50% for VII-A, 45% for X-C and 44% for X-A in Case 1, 55%, 59%, 38% and 36% in Case 2, all 100% in Case 3, 100%, 100%, 66%, and 66% in Case 4, and 100%, 92%, 88% and 100% in Case 5. Deficiency of about 50% level of VII-X in Cases 1 and 2 suggests that the structure genes are located on the long arm of chromosome 13. However, Case 3 with the same karyotype shows the normal results. Thus, following possibilities remain: 1) There are other genes such as regulation genes that contribute to manifestation of VII-X. 2) The karyotype of Case 3 is different from that of Cases 1 and 2. That is, it is an interstitial deletion of 13q, leaving the subtle terminal portion where the structure genes of VII & X are located.

III-23. ***De novo*** **Pericentric Inversion of a Chromosome 13: Ryozo KASAI** (Asahigawa Jidoin Child. Hosp., Okayama), **Kouji NARAHARA, Kiyoshi KIKKAWA, Shunsuke KIMURA and Hiroshi KIMOTO** (Dept. Pediatr., Okayama Univ., Okayama)

The proband, 18 year 8 month-old male, has been institutionalized since 8 years of age because of mental retardation. He was born after 38 weeks of gestation as the second child to an unrelated 30-year-old mother and a 32-year-old father. The delivery was complicated by asphyxia, the birth weight being 3,480 g. Episodes of convulsion appeared since 6 years 10 months of age. Physical examination revealed growth retardation, profound mental retardation, and the following abnormalities: narrow forehead, prominent occiput, arched eyebrows, hypertelorism, saddle nose, wide nasal tip, short philtrum, large mouth, kyphosis, pectus carinatum, cardiomegaly, clinodactyly of 5th fingers, and tapering fingers. Dermatoglyphics of the patient included right simian line, left sydney line, absence of the left digital triradii d, and left high axial triradii (t'). Cytogenetic studies using high-resolution banding techniques showed a karyotype of 46,XY,inv(13)(p13q32.3). The Q-, C-, and N-banding patterns of the inverted chromosome were compatible with the interpretation that one of the breakpoints was located at the telomere of the short arm of a chromosome 13. DNA replication study using BrdU incorporation revealed normal replication of the inverted chromosome 13. The blood coagulation factor VII and X, which are presumed to be located on 13q34 (Pfeiffer *et al.*, 1983), were found to be within normal range (VII and X, 101% and 76%, respectively). These findings suggest that the telomere of the short arm of acrocentric chromosomes may also be involved in structural rearrangements, and that the phenotypic abnormality found in the patient could be caused by either position effects, or by gene mutation at the breakpoints.

III-24. **Origin of the 15q11–12 Deletion in Prader-Willi Syndrome: Tomoko HASEGAWA, Kiyomi YAMADA** (Div. Genet., Natl. Med. Cent., Tokyo), **Eiji KITAZUMI, Shoko ENOMOTO, Akiko YOKOCHI** (Dept. Pediatr., Rehab. Cent. Disabl. Child., Tokyo), **Tomoichi IMAIZUMI, Noriko OKADA, Kazue HIROSE, Makiko OSAWA, Yukio FUKUYAMA** (Dept. Pediatr., Tokyo Women's Med. Sch., Tokyo) **and Masayuki AOYAMA** (Omiya Municipal Gen. Cent. Physic. Ment. Ordit. Handic., Saitama)

Seven cases of Prader-Willi syndrome showing *de novo* interstitial deletion of chromosome 15q11–12 were studied as to parental origin using Q-heteromorphism. Two of them were males and five were females. Every case was studied by high-resolution G- and Q-banding techniques. The elongated chromosomes were obtained by adding ethidium bromide to peripheral lymphocyte cultures before harvest. Six cases of typical Prader-Willi syn-

drome showed a paternal derivation, but one female case with extremely severe psychomotor retardation seemed to be maternal in origin, though the results were somewhat obscure. Several previous reports have suggested preferential paternal derivation of the deleted chromosome 15 in Prader-Willi syndrome. The results in the present cases were consistent with those from other previously reported cases.

III-25. Paternal Origin of the Extra Dicentric Chromosome 15 in Prader-Willi Syndrome: **Tomoko HASEGAWA** (Div. Genet., Natl. Med. Cent., Tokyo), **Hiroko FUJITA** (Dept. Child Health, Osaka City Univ., Osaka), **Kohtaro YAMAMOTO** (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

A case of Prader-Willi syndrome previously reported by Fujita *et al.* (*Hum. Genet.* **55**: 409, 1980) was cytogenetically re-examined. High-resolution G-banding patterns obtained by the treatment with ethidium bromide and by the ASG technique using EB virus-transformed B lymphocytes demonstrated no interstitial deletion on chromosome 15 homologues. The small extra chromosome previously identified as an isodicentric chromosome 15 was also studied by the high-resolution Q-banding technique. Both short arms of this chromosome revealed a similar pattern of Q-heteromorphism. By comparison with the normal chromosome 15 from the parents a paternal derivation of this abnormal chromosome was indicated. In another case of Prader-Willi syndrome reported by Wisniewski, an extra dic(15) was also paternal in origin. Considering the preferential derivation of the deleted chromosome 15 in Prader-Willi syndrome, the occurrence of breakage on the proximal part of chromosome 15 may not be rare in male spermatogenesis.

III-26. A Case of Partial 18q Trisomy Resulting from a Pericentric Inversion of Maternal Chromosome 18: **Tamiko SHINOHARA** (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent., Tokyo), **Tatsuro IKEUCHI** (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Ken ASANO, Kiyoshi HASHIMOTO, Yutaka UEDA** (Dept. Pediatr., Nippon Med. Coll., Tokyo) **and Takeo OGOSHI** (Dept. Pediatr., Shimodate Municipal Hosp., Ibaragi)

The propositus was born at 37 weeks' gestation, on June 8, 1982, as a second child of a 30-year-old mother and a 32-year-old father, who were both phenotypically normal. There was consanguinity on the mother's pedigree, *i.e.* mother's parents were cousins. The mother had two spontaneous abortions and one stillborn. His elder sister is phenotypically normal. Birth weight was 2,890 g. At 2 weeks, his weight was 3,070 g and his length 50 cm, and the following abnormalities were observed: slight frontal bossing, prominent occiput, asymmetrical palpebral fissures, ptosis, large and low-set ears, upturned

nose, small mouth, high arched palate, micrognathia, microgrossia, a simian crease on the left hand, camptodactyly of both fifth fingers, typical overlapping of two medial fingers by the index and the fifth fingers, lack of the 12th costal bone on the right side, and micropenis. Chromosome studies were carried out on peripheral blood lymphocytes and skin fibroblasts. The patient's karyotype was 46,XY,18p+. His mother was found to have a pericentric inversion of chromosome 18. With high-resolution G-bands obtained by the ethidium bromide pretreatment technique, his mother's karyotype was identified as 46,XX, inv(18)(p11.2q21.3). The abnormal chromosome found in the propositus may be interpreted as a recombinant which resulted from a crossing over occurring within the inverted segment. He received a chromosome 18 with a duplication of the segment q21.3→qter and a deletion of the terminal segment pter→p11.2. Thus, the patient's karyotype could be designated as 46,XY,rec(18),dup q,inv(18)(p11.2q21.3)mat., indicating that she is trisomic for q21.3→qter and monosomic for pter→p11.2.

III-27. A Case of 22 Partial Tetrasomy: Misao NAKAJOH,[1] Isamu YONEMURA,[1] Hayato HASEKURA,[1] Yoji HARA[2] and Koichro TSUJI[2] ([1]Dept. Legal Med., [2]Dept. Pediatr., Shinshu Univ. Sch. Med., Matsumoto)

The patient was a seven month old female, born after 37 weeks and 5 days of gestational period to a mother of 34 years of age and a father of 35; the birth weight was 4,400 g. She had tachypnea, cyanosis, anoxia and polycythemia, and the following anomalies: relaxation of muscle, down slanting palpebral fissures, flat dorsum nasi, low-set ears, preauricular skin tags, ear fistula, and unbalanced size of right and left ventricles. Coloboma of the iris was not observed. No abnormality was observed in biochemical examinations of serum. Her dermatoglyphics were examined on palms and soles. Fingerprints from the thumb to the little finger were W, U, U, W, U on the left hand and W, W, U, W, U on the right. Palmprints revealed both main lines A ending in No. 1 area and bilateral loop thenar patterns. The left toe-prints were F, F, F, F, A, and the right were F, F, W, F, A. The karyotype was 47, XX,+mar, possessing a small dicentric chromosome. Karyotypes of the parents were normal. The marker chromosome showed two dark bands by G-banding technique, large and small blocks by C-banding. Satellite associations were observed on one end but not on both ends. A large NOR band was observed on one end and small one on the other end by Ag-NORs staining. This marker was not stained by DA/DAPI staining and probably did not derive from chromosome 15. From these findings, it was inferred that the karyotype was 47,XX,+t(22;22)(pter→cen→q12::q12→cen→p12), namely, the marker chromosome had short stalks and the deletion of satellite on one end.

III-28. Psychological Study of Children with Sex Chromosomal Abnormalities: Hisashi KAWAI (Dept. Psychol. Psychiat. Res. Inst. Tokyo, Tokyo), **Makoto HIGURASHI, Tatsuya TAKESHITA** (Dept. Health Sci., Yamanashi Med. Coll., Yamanashi), **Masaya SEGAWA** (Segawa Neurol. Clin. Child., Tokyo), **Shinzo EGI, Fumihiko TANAKA** (Dept. Pediatr., Univ. Tokyo, Tokyo), **Kumiko TAKADAYA** (Dept. MCH, Univ. Tokyo, Tokyo) **and Kiyohiko KATO** (Dept. Pediatr., Yamanashi Med. Coll., Yamanashi)

This study on children with Turner syndrome, Klinefelter syndrome and XYY was designed to analyze the psychological findings. The subjects were 5 girls with Turner syndrome (11y–16y), one case of Klinefelter syndrome (3y 4m) and two cases with XYY (1y 5m and 7y 3m). As the numbers of the patients were limited, and as the intellectual levels of the patients were in wide range, the generalizations of the findings were difficult. However, relatively common features were found as follows. 1) Turner syndrome. 1. Low responsiveness to emotional stimuli. 2. Poor emotional expressions. Or if any, lacks undulations. Therefore the patients often seem to be a stable, quiet person. 3. Passive, negative mental attitudes. 4. Low mental activity (includes psychological one) and low productivity. 5. Narrower and limited interests. 6. Passive and common-sense adaptability. 7. Lower impulsiveness and aggressiveness. 8. To show little aggressiveness in frustration, express little discouragement. 9. To show little aggressiveness nor apologize rightly when criticized. 10. Passive in personal relations, and stable, lacks interactions. 11. Wide range of the IQ, from subnormal to normal. 12. The patients adapts according to their IQ levels. 13. No psychopathological findings. 2) Klinefelter syndrome. It is impossible to get a general characteristics of this syndrome because of a limited number of cases. The development of the cases of Klinefelter syndrome was in normal limits, and no adaptation problem was found.

III-29. Hypothesis on XYY: Shinichiro NANKO (Dept. Psychiat., Teikyo Univ., Tokyo)

The frequency of XYY males among general population is estimated approximately 0.1%, so that about 50,000 XYY males are supposed to be living in Japan. How are they managing? In the former study, the author indicated that XYY males have the high activity level on personality traits and this trait plays the part of abnormal behaviour on XYY males. Based on the fact that intelligence on XYY males found among general population is intact as far as the recent newborn and general adult population surveys are concerned, while that among penal population is involved, the author suggests that XYY males with impaired intelligence have a tendency to commit a crime especially violent nature with the presence of high activity level. How about the XYY males with normal or high

intelligence level. Characteristic features on these XYY males have been still unknown. According to the above mentioned context, with the presence of high activity level, to say aggressivity in a good sense, they are suspected to be a talented, and have a chance to get a good job. The survey on XYY males among talented population is thus clearly needed.

III-30. 過剰な二動原体性 15 番染色体を伴った Klinefelter 症候群：宮沢裕子・大沢真木子・中田恵久子・福山幸夫（東女医大・小児），**長谷川知子**（国立病院医療センター・遺伝）．**Klinefelter Syndrome with an Extra dic(15):** Y. MIYAZAWA, M. OSAWA, E. NAKADA, Y. FUKUYAMA (Dept. Pediatr., Tokyo Women's Med. Coll., Tokyo), T. HASEGAWA (Clin. Res. Inst., Natl. Med. Cent., Tokyo)

Klinefelter 症候群と autosomal aneuploidy の合併する頻度は，偶然の確率より高いといわれている．一方，extra dic(15) は，それ自体まれな異常であり，Klinefelter 症候群との合併例の報告はほとんど見られていない．今回われわれは，このような症例を経験したので，文献的考察を加えて報告する．症例：1984 年 4 月 23 日出生．母 46 歳，父 28 歳，第 2 子．在胎 39 週，帝王切開，体重 2,830 g，身長 47 cm，頭囲 34 cm，胸囲 33 cm，仮死なし．哺乳力弱い．生後 9 日低体温のため保育器収容．定頸 7 か月．追視 10 か月，座位不可能．身体発育正常，前頭部突出，鼻背低形成，両眼開離，大角膜，高口蓋，筋緊張低下，手掌紋異常を認めた．CT 上中等度脳萎縮，脳波異常を認め，全身強直性けいれん，点頭てんかんをきたした．染色体所見：患児末梢血リンパ球を用いた染色体 G-バンド分析により 48,XXY,+mar の核型を得た．さらに高精度分染法と DA-DAPI バンド法を用いて検索したところ，marker 染色体は両端に satellite を持つ dic(15) (pter→q13 :: q13→pter) であり，15 (pter → q13) の部分が tetrasomy になっていると考えられた．両親の核型は正常．なお extra dic(15) はときに Prader-Willi 症候群に見られるが，本症例では臨床上 Prader-Willi 症候群の診断はつけがたい．これまでに報告された dic(15) では大きな外表奇形を伴わず，精神運動発達遅延，けいれん，筋力低下を認めることが特徴である．

III-31. 低身長を伴った 45,X/46,XY 男子の 3 例：仲田教子・石飛和幸・重白啓司・星野映治・佐々木孝夫（鳥取大・医・三内）．**Three Male Cases of 45,X/46, XY Mosaicism with Short Stature:** Noriko NAKADA, Kazuyuki ISHITOBI, Keiji SHIGESHIRO, Eiji HOSHINO and Takao SASAKI (Dept. Med., Tottori Univ., Yonago)

45,X/46,XY モザイクは X 染色質陰性で，その表現型は外見的に正常男性から性別不詳，不妊女性と大幅な変異を示し，臨床像もターナー症候群，性腺発育不全，仮性半陰陽，混合性腺発育不全などが知られている．われわれは，低身長を主訴とした non-fluorescent Y chromosome をもつ 2 例を含む 3 例の男子に 45,X/46,XY モザイクを認めた．症例 1：初診時年齢 10 歳 5 カ月，身長 117.8 cm (-3.2σ)．耳介低位，左第 4, 5 中手骨短縮あり．停留睾丸はない．染色体核型 45,X/46,XY (30 : 70)．症例 2：3 歳 7 カ月，身長85.1 cm (-3.1σ)．左停留睾丸あり．核型 45,X/46,XY (38 : 62)．症例 3：24 歳，身長 150.5 cm (-3.6σ)．二次性徴の発現を認めるが，小睾丸，無精子症．核型 45,X/46,XY (54 : 46)．Y 染色体の長さは 3 例ともに正常であったが，Q-band 法を行った症例 1, 3 の Y 染色体

に蛍光が見られなかった．また C-band 法を行った症例 1 は，Y 染色体の長腕が濃く染まらなかった．文献上，Q-band 法を施行された 45,X/46,XY 症例はわれわれの症例を含めて 15 例あり，non-fluorescent Y の症例は 12 例報告されている．これらの表現型は男性 4 例，女性 8 例であった．H-Y 抗原は，表現型女性の 2 例に施行され 2 例ともに（−）であった．

III-32. A Female Patient with Two Kinds of Marker Chromosomes and a Clinical Picture Compatible with Turner's Syndrome: **Hiromi SAKAMOTO,[1] Chiharu TAKADA,[1] Miyako YABUKI,[1] Osamu MIKAMI,[1] Yoshihiro YAMAMOTO,[1] Jun-ichi FURUYAMA[1], Norimitsu OHTSUKA,[2] Yoshie SUGAHARA,[2] Satoko YOKOTA[2]** ([1]Dept. Genet., [2]Dept. Clin. Lab., Hyogo Coll. Med., Nishinomiya), **Osamu NOSE, Tokuzo HARADA, Ichiro MAKI and Saburo KIMURA** (Dept. Pediatr., Osaka Univ., Osaka).

An 11-year-old girl was evaluated for short stature. Her parents and two elder brothers showed normal height. There was normal spontaneous delivery following a normal pregnancy. Just after birth, her sucking was not good, but no remarkable developmental retardation could be pointed out when she was one year old. When she became 10 years old, her short stature was notorious. Her hight was 119.9 cm. No secondary sex characters had developed. She had cubitus valgus, webbed neck, hypoplastic nails, low hairline, and many nevi on the face and forearms. Her total finger ridge count was 226. Endocrinological examination showed the following: LH and FSH were high, HGH was low, TSH was normal levels. Radiograph of hand showed a little delay in skeletal maturity. Chromosomal analysis was made on peripheral blood lymphocytes using various banding methods: GTG, QFQ, RBG, CBG, and high-resolution G banding. Her karyotype was identified as mos 45,X/46,X,del(X)(p11q24)/47,X,del(X)(p11q24),del(X)(p11q22).

III-33. A 14-Year-Old Girl Showing Mosaic X/XY, Trisomy 18 and Y/Autosomal Translocation: 45,X/47,XY,−19,+t(Y;19)(q12;p13),+18: **Shizuhiro NIIHIRA, Hiroko FUJITA** (Dept. Child Health, Osaka City Univ., Osaka), **Takuma KONDO,[1] Mamoru NAGANO[2]** ([1]Dept. Med., [2]Lab., Osaka City Child Health, Osaka), **Norimitu OTZUKA[3] and Tomoko HASHIMOTO[4]** ([3]Lab., [4]Dept. Genet., Hyogo Med. Coll., Nishinomiya)

A 14-year-old girl with short stature and mental retardation was investigated. Cytogenetic findings: G banding analysis on both T-cells (stimulated by PHA) and B-cells (by EB virus) of peripheral blood revealed two cell lines of 45,X and 47,XY,19p+,+18. According to Q-banding on the 19p+, translocation of a portion of the long arm of Y to the short arm of chromosome 19 was disclosed. The cultures of skin fibroblast showed only 45,X cells. Physical findings: She presented many stigmata of Turner syndrome such as short stature (−6.3 SD), low posterior hair line, broad chest with widely spaced nipples, cubitus valgus

and lack of second sexual development. However, she exhibited no features of 18 trisomy except for short sternum and severe growth and mental retardation. Hormonal examination: Level of LH, FSH were extremely elevated and abnormal response to LH-RH was shown. Estradiol was 9.9 pb/ml (control 16–130) and testosterone 41.8 ng/dl (control 18–37). She is suspected of having a gonadal dysgenesis. Data of laparotomy: A hypoplastic uterus with oviducts, round ligaments and small gonads was located in the position of female internal genitalia. Histological examination of the gonads demonstrated a streak apperance in left side and dysgenetic seminiferous tubules and ovarian interstitial cells without follicular cysts. Therefore diagnosis from histology was mixed gonadal dysgenesis. To our knowledge, there has been only two cases of mosaic 45,X/47,XY,+18 in the literature (Schinzel *et al.*, 1974 and Serville *et al.*, 1977).

III-34. A Case of 45,X/46,XXp+: Hideki TERAMOTO, Masaaki TAKENAKA, Kazushi NOMURA, Koso OHAMA and Atsushi FUJIWARA (Dept. Obstet. Gynec., Hiroshima Univ., Hiroshima)

A 14-year-old girl was referred for evaluation of shortness of stature. She was born at term after an uneventful pregnancy, with weight of 2,850 g. Main clinical findings were as follows: short stature (140.9 cm), primary amenorrhea, webbed neck, shield chest, bilateral epicanthus, right blepharoptosis and infantile genitalia. Axillary and pubic hair were absent and there was no cubitus valgus. Serum FSH and LH levels were elevated, serum E_2 level was low, and serum GH level was normal. X chromatin analysis from buccal smear showed 74% of 100 cells to contain a large, bipartite or separated X bodies. Chromosome analysis in peripheral blood lymphocyte cultures revealed the presence of two cell clones. 20% of the cells had a karyotype of 45,X, while the remaining 80% had a large submetacentric chromosome with loss of one X chromosome. G-, Q-, R- and C-banding analyses indicated that the abnormal chromosome was derived from two X chromosomes joined by their distal short arms at bands p22, and that the rearranged X chromosome was always late replicating. The chromosome constitution of the patient revealed 45,X/46,X, psu dic (X) ter rea (X;X)(qter→cen→p22::p22→qter). Karyotypes of the parents were normal. There were 24 reported cases showing an identical karyotype with ours. Their stigmata of Turner syndrome were mild as a whole.

III-35. 原発性無月経を主訴とした 45,X/46,X,+mar の 1 例について：林美貴子・本田幸子（富山県衛生研），**井本正樹**（井本産婦人科医院），**藤田弘子**（大阪市大・生活科学）．**A Case of 45,X/46,X,+mar with Primary Amenorrhea:** M. HAYASHI, S. HONDA (Toyama Inst. Health, Toyama), M. IMOTO (Imoto San-Fujinka Clin., Toyama) and H. FUJITA (Osaka City Univ., Osaka)

25 歳の原発性無月経の女性の染色体検査を行ったところ，45,X と 46,X,+mar のモザイクであった．症状は低身長 (142 cm)，短軀，短頸，外反肘，乳腺発育不良，小さい子宮，少ない陰毛，第II度無月経がみられた．LH-RH テストからは卵巣不全型と診断された．性染色質は陰性であり，マーカー染色体をもつ細胞の割合は 58.5% であった．マーカー染色体は比較的大きな端部動原体型であり，C バンドで 2 個所濃染し，Q バンドでも同じ個所が強い蛍光を発し，また，休止核では比較的大きな F-body がみられた．R バンドでは両端と中央に比較的よく染色される部分があり，5-azacytidine 添加培養 (2×10^{-7} M, 7 時間) でも R バンドでうすく染色される部分がよくのび，Y 染色体の異常が考えられた．さらに，Schmid ら (1984) の例と比較し，各分染法と併せ，マーカー染色体は dic(Y) (pter → cen → q12 :: q12 → q11 :: q11 → q12 :: q12 → pter) と推定された．

III-36. Cytogenetic Studies in a Female Infant with Proposed Karyotype of 45,X/46,X,i dic(X)(p22::p22): **Yoshifumi YAMAMOTO** (Dept. Pediatr., Jichi Med. Sch., Tochigi), **Norio SUZUKI,[1] Shiroh MATSUYAMA[1] and Takenobu KOIZUMI[2]** ([1]Dept. Surg., [2]Dept. Neonatol., Gumma Child. Med. Cent., Gumma)

A newborn female with anal atresia, duodenal perforation and mild Turner stigmata was found to have a presumptive karyotype of 45,X/46,X,i dic(X)(qter→p22::p22→qter) in her lymphocytes and skin fibroblasts. The rearranged chromosome had only one primary constriction and always showed late replication. The constitutive heterochromatin at the pseudocentromeric region was C-band positive but Cd-band negative. Analysis of DNA replication sequence by B-pulse methods in leukocytes showed more than two types of sequence at the distal long arm of the i dic(X). The sequence was variable among different cells, but seemed to be almost stable within each cell. In two cells, however, the sequence at both arms was different from each other. The DNA replication sequence spreading from near centromere to distal long arm may not be strictly related. The pattern of DNA replication did not relate to the position of active centromere.

III-37. ダウン症候群の皮膚紋理分析—数量化理論を用いて—：山田一朗・浅香昭雄（東大・医・保健），**松井一郎**（愛知コロニー・発達障害研）．**Analysis of Dermatoglyphics of Down Syndrome Using the Method of Quantification:** Kazuaki YAMADA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo) and Ichiro MATSUI (Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

ダウン症候群の各種皮膚紋理の出現頻度は，健常者とは異なった分布を示すことが知られている．このことを用いて，皮膚紋理所見によるダウン症候群と健常者群との鑑別診断の試みがすでにいくつ

か報告されている．これらの報告の多くは，ある特定の皮膚紋理の出現頻度をダウン症候群と健常者との間で比較し，その違いをなんらかの形で指標化することを解析の出発点としている．今回われわれは，ダウン症児 251 名（男子 140 名，女子 111 名，核型はいずれも 21 trisomy），およびコントロール群として健常学童 1,008 名（男子 514 名，女子 494 名）を対象とし，どのような紋理がダウン症に特徴的であるかを総合的に考察するための指標を数量化II類を用いて作成した．

分析に用いた項目は，性別，10 指の紋理型（A, U, R, W の 4 分類），軸三叉の位置（t, t′, t″ の 3 分類），猿線，第 5 指単一屈曲線（あり，なしの 2 分類）および，足の母指球紋理（A^t, L^d, L^t, W, その他の 5 分類）である．各項目に与えられた重み係数の総和が正の値となるとき健常者を，負の値のときダウン症を示すように設定して計算した．判別に寄与する大きな負の重み係数が与えられたのは，右足母指における A^t の出現（−0.57），右手猿線（−0.55），左手第 5 指における R の出現（−0.54），左手猿線（−0.51）などであった．各個体別に重み係数の総和を計算し，判別の的中率を求めたところ，健常者では 91.3%(920/1,008)，ダウン症では 98.0%（246/251），全体では 92.6%（1,166/1,259）となった．

III-38. 日本人小児の頭部，手の各部位の計測値：五十嵐美絵・梶井　正（山口大・小児）．**Normal Ranges for the Head and Hand Measurements in the Japanese Children:** Mie IGARASHI and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

0～15 歳小児 1,800 人について，頭部，手の次の 20 部位の計測を行った：1）内眼角幅，2）外眼角幅，3）瞳孔間距離，4）鼻長，5）鼻幅，6）人中の長さ，7）8）口唇の厚さ（上，下），9）口裂幅，10）頬骨弓幅，11）最大頭幅，12）最大頭長，13）頭囲，14）全頭高，15）顔面高，16）第 5 指長，17）手掌長，18）第 3 指長，19）耳長，20）基準線より上方の耳長．各年齢群男女それぞれ 50 名について平均値，正常範囲（±2SD）を求めた．2 歳男児の内眼角幅の平均値 ±2SD は，3.0±0.48 cm で，3.48 cm 以上をいちおう両眼開離と診断してよいと考える．小児の頭部，手の計測値は先天性奇形の診断に欠くべからざるものだが，日本人小児の計測値は，二，三の項目以外は報告がない．白人小児については各種の計測値の報告があるが，人種差があるために日本人小児には適用しえない．本研究ではこのギャップを埋めることを試みた．

III-39. Overgrowth syndromes の鑑別：塚原正人・梶井　正（山口大・小児）．**Differential Diagnosis of Overgrowth Syndromes:** Masato TSUKAHARA and Tadashi KAJII (Dept. Pediatr., Yamaguchi Univ. Sch. Med., Ube)

Overgrowth syndromes の 12 例（1）Sotos 症候群 8 例，2）Beckwith-Wiedemann 症候群 1 例，3）Golabi-Rosen 症候群 1 例，4）不明 2 例）について検討した．Overgrowth syndromes に共通する臨床所見は出生前に始まる身長・頭囲・体重の過多，骨年齢の促進，精神遅滞，種々の奇形である．少なくとも 17 種の疾患が知られているが，その病因は，diabetic macrosomia, X 連鎖劣性遺伝の形式を示す Golabi-Rosen 症候群，Simpson dysplasia 症候群，およびその他 2, 3 を除いて明らかでない．鑑別診断の決め手となる検査所見が乏しいので，頭蓋，顔面奇形，内臓奇形，骨異常などの臨床所見を総合的に判断せざるをえない．しかし，臨床所見は同一疾患でも個々の症例により差異が

あり，異なる疾患群の間で重複することがあり，鑑別が困難であることが多い．頭蓋，顔面奇形から派生する顔貌が鑑別上とくに重要である．Sotos 症候群と Beckwith-Wiedemann 症候群は特有な顔貌を示す．Golabi-Rosen 症候群では，顔貌に加えて骨異常・内臓奇形に特徴がある．Sotos 症候群では一般に内臓奇形はないが，4 例に従来報告のない先天性心疾患（心房中隔欠損，心室中隔欠損自然閉鎖，僧帽弁閉鎖不全，三尖弁閉鎖・肺動脈狭窄）を認めた．いずれも心疾患合併にもかかわらず過度発育を示した．Sotos 症候群 5 例について尿路の奇形を検索し，1 例に右腎欠損，1 例に膀胱尿管逆流現象および尿管拡張を認めた．

III-40. 全身的な hypertrichosis を伴った idiopathic gingival fibromatosis の一例：吉森寿美代[1]・古田 勲[1]・荻田善一[2]（富山医薬大・[1]歯口外・[2]和漢研・病態生化）．**Idiopathic Gingival Fibromatosis with Hypertrichosis:** Sumiyo YOSHIMORI,[1] Isao FURUTA[1] and Zenichi OGITA[2] ([1]Dept. Oral Surg., [2]Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama)

今回，われわれは，全身的な hypertrichosis を伴った idiopathic gingival fibromatosis の一例を経験し，病理組織学的検討に加え，細胞遺伝学的解析および電気泳動法的解析を行った．病理学的には，アルシャンブルー染色にて酸性ムコ多糖体の増加を認め，トルイジンブルー染色にて，ヒアルロン酸が主体であることを確認した．本例の末梢血リンパ球の核型解析では，異常は認められなかった．しかし，SCE 頻度では，健常が約 7% であるのに対し，本例は 11% と高値を示した．これは，なんらかの環境変異原の存在を示唆するものと考えられる．歯肉組織の蛋白成分の Triton X-100 system による電気泳動では，健常者に比較し，主画分蛋白成分の減少を認めたが，質的差異は認められなかった．SDS system による比較検討では，主画分の下端に本例に特異的な低分子蛋白画分を認めた．本例および健常者の顎下腺，舌下腺混合唾液の蛋白成分を SDS system により電気泳動法的に比較したところ，大きな質的量的差異は認められなかった．

III-41. Wiedemann-Beckwith Syndrome (WBS): Genetic Analyses of 22 Cases in Four Families: Norio NIIKAWA (Dept. Genet., Nagasaki Univ. Sch. Med., Nagasaki), **Satoshi ISHIKIRIYAMA and Satoshi TAKAHASHI** (Dept. Pediatr., Hokkaido Univ., Sch. Med., Sapporo)

A total of 22 cases of WBS in four unrelated families were reported. Of these, 7 were "definite patients," 12 were "probable patients," and 3 had incomplete features of the syndrome. High-resolution chromosome analyses on the 7 definite patients revealed no abnormalities. Pedigree analyses on the 4 present and 18 reported families revealed the following findings: 1) The sex ratio in patients was 54 : 38 that was not significantly different from 1 : 1. 2) There was a family showing male to male transmission of the trait. 3) There was only one family in which parents were consanguineous. 4) Nine families had several patients in each generation. 5) In some families, both the siblings of patients and those of carriers were affected. 6) The phenotype was variable in not only carriers but also patients. Moreover, there were in some families several individuals with incomplete fea-

tures of WBS who had not been diagnosed as a patient or a carrier. 7) When patients and carriers are combined together as being affected, a ratio of affected to non-affected would be 1 : 1. 8) A segregation ratio in the siblings of patients is 0.461 ± 0.044 (sib method). These findings indicate that the condition in the 22 families is compatible with autosomal dominant inheritance with variable expressivity.

III-42. Extremely Complicated Balanced Reciprocal Translocations with Eight Break-Points in a Dysmorphic Infant: Yoshimitsu FUKUSHIMA,[1] Yoshikazu KUROKI[1] and Taizo ITO[2] ([1]Div. Med. Genet., [2]Div. Ophthal., Kanagawa Children's Med. Cent., Yokohama)

The patient, a one-month-old female infant, was born at 39 weeks' gestation to a 32-year-old father and a 30-year-old mother. Her birth weight was 2,800 g. The parents were healthy and were not related. They had no history of taking radiation and drugs. A two-year-old brother was healthy and did not have any malformation. The pregnancy was unremarkable except for a high rubella antibody titer (256×) in the mother at the 3rd month of gestation, although the mother had no history of rubella infection. When first seen by us at the 21th day, her sizes were 49.2 cm in length, 2,950 g in weight and 31.5 cm in head circumference. Following anomalies were noticed; ocular abnormalities including sclerocornea and corneal staphyloma in the left eye, microcephaly, hirsutism, prominent ears with hypoplastic helix, retrognathia, redundant skin around the neck, wide-set nipples, bilateral hypoplastic thumbs, clinodactyly of fifth fingers, syndactyly between right fourth and fifth toes, hypoplastic right third toe, left cleft-foot and abnormal dermatoglyphics with distal axial triradius. Chromosome analysis using G-banding revealed extremely complicated balanced reciprocal translocation with eight break-points. The karyotype was interpreted as 46,XX,t(1;6;7;3;11)(11;22;21)(1qter 1p22::11p15 11pter;6qter 6p21::1p22 1pter; 7qter 7p15::6p21 6pter; 3pter 3q27::7p15 7pter;3qter 3q27::11p15 11q11::21q11 21qter; 22qter 22p11::11q11 11qter;21pter 21q11::22p11 22pter). Examinations and karyotyping of the parents were refused.

IV-1. Clinical Genetic Studies on Benign Familial Hematuria Associated with Sensorineural Hearing Loss: **A. MATSUI,[1] S. TOMIYAMA,[2] T. WATASE,[2] J. YAMAGUCHI,[2] S. BABA,[2] M. KITAGAWA[3]** ([1]Dept. Pediatr., [2]Dept. Otolaryngol., [3]Dept. Ophthal., Isesaki City Hosp., Isesaki), **and T. MATSUDA** (Dept. Anatomy, Toyama Med. Pharm., Univ., Toyama)

Symptomless hematuria is characterized by monosymptomatic hematuria of unknown etiology, neither associated with any progressive renal diseases nor lower urinary tract lesions. It may be divided into two types: 1) familial type in which hematuria is observed in several family members in more than two generations; 2) nonfamilial type in which hematuria is not observed in any other family members except for the proband. The familial type is named benign familial hematuria because of its favourable prognosis. We have already confirmed by statistical method that benign familial hematuria is autosomal dominant with penetrance of more than 93%, and published an article in which benign familial hematuria in Japanese, American, and Israelite was compared from genetic point of view. The present study has been performed to examine if benign familial hematuria is associated with sensorineural hearing loss. Hearing was tested utilizing Audiometer AA-16 BN type of the RION in 111 family members among 50 pedigrees of benign familial hematuria. Sensorineural hearing loss was diagnosed when threshold of bone induction was raised to more than 25 dB in hearing level. Sensorineural hearing loss was observed in 16 family members (14.4%) among 16 pedigrees (32%) of benign familial hematuria. High-frequency sensorineural hearing loss was observed in 10 individuals (unilateral 7, bilateral 3) and unilateral low-frequency one in 3. Sensorineural hearing loss in all frequencies was observed in 3 (unilateral 1, bilateral 2). Thus, some cases of benign familial hematuria were found to be associated with sensorineural hearing loss. Those cases of benign familial hematuria associated with sensorineural hearing loss may occur in consequence of pleiotropic gene expression in benign familial hematuria or that in Alport's syndrome. Alternatively, these two clinical entities may be induced by the same gene, which manifests Alport's syndrome in the most severe form, benign familial hematuria in the most benign form, and benign familial hematuria associated with sensorineural hearing loss in the intermediate form.

IV-2. A Case Report of Thrombocytopenia with Absent Radius (TAR Syndrome) Associated with Marker Chromosome: **Hideo TAKI, Hiroshi TAMAI, Miki TOKUHIRO, Yuko KOKUI, Seiichi SHIMADA, Masahisa FUNATO** (Dept. Pediatr., Yodogawa Christian Hosp., Osaka) **and Mutsuhiro FURUTA** (Dept. Pathol., Natl. Kyoto Hosp., Kyoto)

The radial aplasia-thrombocytopenia syndrome was first described in 1929 by Greenwald and Sherman, and was named TAR syndrome by Hall *et al.* More than 150 cases have

been reported in the world literature by 1982. We here report a further case. A female infant was born at term to a 23-year-old father and a 21-year-old mother. She was brought to our hospital immediately after birth because of the deformities of the hands. She was the first child of this unrelated parents. The pregnancy was uncomplicated and the delivery was uneventful. The mother denied usage of drugs, exposure to X-rays and toxins during pregnancy. The birth weight was 2,410 g. There was no history of congenital defects in the family. She died on the 20th day of life despite intensive care. She had the following congenital abnormalities: 1) Short stature. 2) The right arm was shorter than the left, and both hands were radially deviated. The thumbs were present. 3) Micrognathia. 4) Low set and deformed ears. 5) Left corneal opacity. 6) High arched palate. 7) Single umbilical artery. 8) High placed umbilicus. 9) Sacral dimple. 10) Congenital heart defects (VSD, AS, PDA). The following findings were revealed by hematologic, radiographic and immunological examinations: moderate leukocytosis, thrombocytopenia with hypoplasia of megakaryocytes, aplasia of right distal radius with shortened and bended ulna, hypoplasia of left radius, IgM 22 mg/dl, IgA 0 mg/dl, and IgG 835 mg/dl. The cytogenetic analysis revealed the karyotype: 46,XX,−22,+mar. Both parents have not been tested yet.

IV-3. A Case Report of Multiple Anomaly Syndrome Related to Maternal Tobacco Smoke Exposure: Hideo TAKI, Mayumi YAMAMOTO, Hiroshi TAMAI, Seiichi SHIMADA and Masahisa FUNATO (Dept. Pediatr., Yodogawa Christian Hosp., Osaka)

A six-week-old male infant was referred because of congenital deformities. He was born at term to healthy unrelated parents, weighed 1,530 g (maternal age 30 y. o., paternal age 29 y. o.). He was the first baby for the parents. The pregnancy was uneventful except maternal tobacco smoking. There was no history of medication. He had the following congenital abnormalities: 1) prenatal onset growth deficiency; 2) hypotonia; 3) microcephaly; 4) severe hypoplasia of skull; 5) wide fontanels; 6) broad nasal bridge; 7) upsweep of frontal scalp hair; 8) low set ears; 9) micrognathia; 10) protruded lower lip; 11) relative shortness, especially of forearm; 12) hypoplasia of fingers, toes and nails; 13) midline occipital protruded verruca. Now, he is 1 year and 4 month old. Coarse gray coloured skin, lack of subcutaneous fat, splenomegaly and hypertrophied muscle are pointed out. His developmental milestones were severely delayed: head control 1y. 2m.; DQ 38 (1y. 4m.). TORCH titer was normal. Hyperlipidemia of Type V was present (T-TG 767 mg/dl, phospholipid 205 mg/dl, betalipo 681 mg/dl, NEFA 2.7 mEq/*l*, T-CHO 213 mg/dl, E-CHO 142 mg/dl, alpha-lipo 14.3%, pre-beta 26.6%, beta 34.6%, tailing 13.4%, chylomicron 11.1%). UA (11.1 mg/dl), insuline (6.7 μU/ml) and glucagon (170 pg/ml) were

normal level. His karyotype was normal. An association between maternal smoking and low birth weight has been reported in many instances, whereas reports of a relationship between maternal smoking and congenital defects are contradictory. A significant association was found between smoking during pregnancy and increased incidence of cleft lip and pelate in the offspring. In another report anencephaly was associated with smoking. Several other studies, however, have not supported the idea of smoking as a cause of congenital malformations. Our case has many congenital abnormalities and might be related to maternal tobacco smoking. This patient has a strong resemblance to the patient with abnormality due to aminopterin and with general lipodystrophy.

IV-4. A Case Report of Absence of Tibia with Polydactyly: Kenji NARITOMI, Akihiko KODAMA, Kohji SAMESHIMA and Tamotsu TERAWAKI (Dept. Pediatr., Kagoshima Univ., Kagoshima)

The patient was a 23-day-old boy who was the first child of healthy parents with complicated consanguinity. He was delivered at 36 weeks' gestation by breech presentation. Birth weight was 2,400 g. He was referred to our clinic because of multiple skeletal malformations and cyanosis. Physical examination revealed bilateral hypoplasia of knee and foot joints. Knee joints were abnormally kept at flexed position. Right foot joint was flexed medially and left foot joint laternally. Two digits were present in right foot, and 3 digits in left foot. Roentgenogram revealed bilateral absence of tibia and first and second rays of foot finger, ectrosyndactyly of third and fourth digits of right foot, and syndactyly of third and fourth digits of left finger. Preaxial polydactyly was present in left thumb, and right thumb was thin and fixed distally. His countenance was similar to conotrunchal face. Grade 1/6 systolic murmur was ausculated in the third intercostal space. ECG revealed right axis deviation, right atrial dilatation, and right ventricular hypertrophy. Though cyanotic congenital heart disease was suggested, further examination could not be done because of progressive cardiac manifestation. He died of cardiac failure on the next day of his visit. Autosomal recessive mode of inheritance was suggested because of consanguinity.

IV-5. ラインハルト型，肢中部異形成の一例：木田盈四郎・上原真理子（帝京大・医・小児），**鶴田登代志**（三重大・医・整形）. **A Case of Mesomelic Dysplasia, Type Reinhardt:** M. KIDA, M. UEHARA (Dept. Pediatr., Teikyo Univ. Sch. Med., Tokyo) and T. TSURUTA (Dept. Orthop., Mie Univ. Sch. Med., Tsu)

小人症と全身の骨異常を主訴として診察を希望した 7 歳 1 カ月の女児．身長 91 cm (−5.8SD)，体重 16 kg (−1.9SD) で外見上，四肢長管骨は太く短く，両外反足，手指は短縮し関節の過伸展を認めた．父は 29 歳，母は 25 歳のときに患児が生まれ，同胞には 9 歳の兄があるが異常はなく，家

族歴には，その他特別のものはなかった．妊娠中に悪阻が強く，ブドウ糖とグルタイド 200 mg の注射を最終月経後 30 日と 31 日の 2 回受けている．分娩は骨盤位で，生下時体重は 3,050 g であった．レ線所見では，上肢の中間肢節（前腕）の短縮，とくに尺骨遠位の低形成と橈骨骨幹の橈側凸湾曲，近位橈尺関節の離開，手根骨核出現の遅滞を認めた．下肢では先天性股関節脱臼と中間肢節の短縮，とくに腓骨近位の低形成が著明で腓骨は細く，遠位で低形成，脛骨は太く短く，骨幹湾曲，内果の著明な形成不良と外反足変形を認めた．本症例は文献上きわめてまれな常染色体性優性遺伝で，本邦の第 1 例と考えられる．

IV-6. Lowe's Syndrome with Splenomegaly: Takashi YAMAIRI (1st Div. Intern. Med., Child. Med. Cent. Osaka City, Osaka), **Akemi TANAKA, Den ISSHIKI** (Dept. Pediatr. Osaka City Univ. Med. Sch., Osaka) **and Chiyo IWAMURA** (Dept. Pediatr., Juso Municipal Hosp., Osaka)

Lowe's syndrome is characterized by congenital cataracts, glaucoma, mental retardation and renal tubular acidosis. Yamashina *et al.* reported an increase of urinary excretion of undersulfated chondroitin sulfate A and an elevation of nucleotide pyrophosphatase activity in cultured skin fibroblasts derived from the patients with Lowe's syndrome. It is suspected that the degradation of active sulfate is increased in those patients. In this report, a boy with Lowe's syndrome, who showed a remarkable splenomegaly and pancytopenia when he visited our hospital at the age of 9 months, was described. He showed not only typical clinical features of Lowe's syndrome but also splenomegaly (13 cm below the costal margin) and pancytopenia. But those findings were rapidly improved in about 6 months during the treatment of oral administration of sodium bicarbonate, citrate, sodium phosphate and 1-α-D_3. At the age of 2 years and 6 months, oral administration of ATP and desiccated thyroid, which were said to stimulate the synthesis of active sulfate, was begun. That therapy showed a little good effect on the muscle hypotonia. Nucleotide pyrophosphatase activities of the patient and his mother were 31.1 and 1.4 mU/mg protein (normal; 3 ± 1 mU/mg protein), respectively. This disorder is usualy inherited as X-linked recessive trait. In enzymatic study in cultured skin fibroblasts, patients with Lowe's syndrome show a remarkable elevation of nucleotide pyrophosphatase activity and the activity of mothers' fibroblasts also elevate about a half value of that of patients'. Our case also showed an elevated activity of nucleotide pyrophosphatase, but his mother did not show a sufficient high activity as heterozygote level. The splenomegaly and the pancytopenia were disappeared by alkali therapy. It is suspected that those were caused by severe metabolic acidosis. In conclusions, our case is unique in respect of the following two points: splenomegaly and enzyme activity. It is probable that Lowe's syndrome may not be a single entity.

IV-7. A Case of Cat Eye Syndrome: Marie NISHIHARA, Yoshimitsu FUKUSHIMA and Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

The so-called Cat Eye syndrome is characterised by typical clinical features including anal atresia, ocular coloboma, preauricular tags of sinuses, congenital heart disease, urinary tract anomalies and mental and physical retardation. The patient was 3,180 g (25th percentile), 50 cm (25th percentile) male newborn infant of an uncomplicated 41-week gestation. This patient was the third child of a 32-year-old mother and 32-year-old father who are healthy and unrelated. Both 6-year-old brother and 4-year-old sister are healthy. There was no family history of birth defects nor mental retardation. After a spontaneous vaginal delivery, the infant was admitted to KCMC (Kanagawa Children's Medical Center) because of imperforate anus. Physical examination revealed hypertelorism, downward slanting of palpebral fissures, micrognathia, low set ears and bilateral preauricular sinuses. Atrial septal defect was found but no coloboma of the iris and fundi was detected. The sigmoid loop colostomy was performed to high imperforated anus without rectovesical fistula at the age of 1 day. The chromosome number was 47 in 100% of the metaphases (50 out of 50 cells). All metaphases had an extra small submetacentric chromosome which was G group size or smaller. Giemsa-trypsin, quinacrin, and centromeric banding was performed. The presence of NOR at the distal end of the long arm of the extra chromosome was demonstrated by silver staining. The same region was negative for DAPI staining. Two centromers were shown by DA+DAPI staining. The karyotype was determined to be 47,XY,+i(22pter→q11). The findings in this case support the proposal by Schinzel *et al.* that the term Cat Eye syndrome should be applied only to cases with risomy or tetrasomy of not more than 22pter→q11 and without additional duplication or deletion of another autosomal segment.

IV-8. The Number of Epidermal Ridge Minutiae on the Palm and Its Genetic Features: M. OKAJIMA and K. USUKURA (Dept. Forens. Med., Tokyo Med. Dent. Univ., Tokyo)

The total minutia count (TMC) was examined in palm prints of 20 pairs each of monozygotic (MZ) and dizygotic (DZ) twins. The mean and distribution of the TMC are 984.4 (752–1,254) for the right and 1,001.5 (726–1,310) for the left palm in males, and 911.9 (705–1,178) and 920.6 (772–1,059), respectively, in females. The bilateral difference is not significant. The correlation coefficient between both palms is 0.866 for males and 0.782 for females. The TMC is apparently higher in males than in females. Then the TMC was compared between twins. The intraclass correlation coefficient obtained from the value of both palms combined presented 0.827 in MZ twins and is higher than 0.313 in

DZ twins. This reveals that the TMC is, to a considerable extent, controlled genetically. The TMC represents the number of epidermal ridges. Therefore, it was compared with the total interdigital ridge count, the sum of a–b, b–c, and c–d. Slight correlation coefficients, 0.227 for the right and 0.182 for the left palm, suggest that the appearance of minutiae is related to the absolute amount of ridges as well as to the average length of ridges.

IV-9. 双生児の疾病自然史に関する研究：秋山尚孝[1]・佐藤幸男[1]・岡本直正[1]・渡辺正治[2]・栗原　登[2]・今村展隆[3]・久住静代[3]・蔵本　淳[3]・務中昌己[4]（広島大・原医研・[1]遺伝，[2]疫学，[3]内科，[4]生物統計），**伊藤千賀子**（広島被爆者健管所）. **Analysis of the Diseases of the Atomic Bomb Exposed Twins:** N. AKIMOTO, Y. SATOW, N. OKAMOTO, M. WATANABE, N. KURIHARA, N. IMAMURA, S. KUSUMI, A. KURAMOTO, M. MUNAKA (RINMB, Hiroshima Univ., Hiroshima) and C. ITOH (ABHCC, Hiroshima)

〔目的〕 遺伝と環境要因のかかわり合いを調べる目的で，原爆に被爆した双生児を対象として，被爆条件がもたらす影響を検討することによって疾病自然史の比較検討を行った．〔症例と結果〕 1) 卵性の判明している，片方または両方が被爆した双生児：一卵性双生児 11 組中，疾病の一致しているのは 2 組，不一致 7 組，正常 2 組で，一致および不一致共に胃の疾患が多く，一致組では Ig 値や嗜好品，被爆条件が類似しているが，不一致組では以上の諸条件はバラツキを示した．また HLA 抗原と関連した疾患は見いだされなかった．以上より一卵性双生児の疾病の一致している組では種々な指標が似ている傾向がみられたが，疾病の不一致例の原因は被爆条件の差によるものではない傾向を示した．2) 卵性の判明していない両方が被爆した双生児：男男 20 組，女女 18 組の計 38 組（異性双生児は除いた）中，疾病の類似 10 組，やや類似（一部の疾患は類似，一部は非類似）12 組，非類似 11 組，正常 7 組で，疾病類似組と被爆 2 km 以内に白血球増多，肝障害が多くみられ，被爆距離 2 km 以内のものに疾病の類似傾向がみられた．3) 2 km 以内で被爆した父または母 (P) とその双生児および同胞 (F_1)：男男 5 組，女女 4 組（異性双生児は除いた）とその同胞男 9 人，女 5 人の中で被爆した父 1 例，母 8 例の疾病はおのおの多様で，P と F_1，F_1 と同胞間および F_1 の双生児間には疾病の類似傾向は認められなかった．

IV-10. A Twin Study on Behavior Characteristics in 3-Year-Old Children: Noboru ODA, Kazuhiko ABE (Dept. Psychiat., Univ. Occup. Environ. Health, Kitakyushu) **and Hiroyuki HATTA** (Dept. Psychiat., Kitano Hosp., Osaka)

From 1969 to 1981, seventy nine pairs of Japanese twins of same-sex were examined at a municipal well-child clinic for 3-year-olds (Abeno, Osaka City), for routine physical and psychological check-up. Zygosity diagnosis was based on one or more of the methods, *i.e.*, physical similarity, finger printing, and blood typing with at least 25 antisera, by which 56 of the twin pairs were classified as monozygotic (MZ) and 15 as dizygotic (DZ). The zygocity for the remaining 8 pairs could not be determined. The data on behavioral characteristics in the twins were obtained by questionnaire answered by the mothers. The

questionnaire included items on developmental and current behavior characteristics. The results of observation and tests by a psychiatrist and psychologists were also used as data. The result of the questionnaire revealed that there were significantly higher concordance rates in MZ twins than in DZ twins on the following items: Age when the twins started to walk without support, tendency to be startled by noises, degrees of fear of strangers in infancy, being able to sleep alone without a parent sitting nearby, eczema, motion sickness, hyperhidrosis, enuresis, and motor dysphasia. On the contrary, no such difference was found among two twin groups in items about daily routines concerning with sleep, food and play habits. With regard to the data objectively assessed by a psychiatrist and psychologists, the concordance rate of MZ twins was significantly higher than that of DZ twins on "finger test" (a test devised to assess eye-hand coordination), articulation, hyperactivity and stranger anxiety during the tests.

IV-11. Gerontological Research on Aging Monozygotic Twins Reared Apart and Together: **Kazuo HAYAKAWA** (Dept. Public Health, Kinki Univ., Osaka)

A twin research has been conducted on 630 twin pairs for the purpose of investigating aging phenomenon from the environmental aspects. The mailed questionnaire survey has been done on all the 630 pairs. 44 out of 630 pairs had a comprehensive medical examination at Kinki University Hospital (ECG, SMAC total blood tests, HB Ab. and Ag., Ig-G.A.M.D.E., MPI, WAIS, OKT monoclonal antibody of lymphocyte membrane antigen, *etc.*). The chronological age of separation, the age when the twin started to live apart, varied from 0 to over 30. In the present study, immunological results have been analysed. Among the 40 monozygotic pairs (aged over 50 years old), intrapair correlation coefficient of serum immunoglobulin level was highest at Ig-M (0.882), followed by Ig-A (0.729) and Ig-G (0.674). Ig-E showed the lowest intrapair correlation coefficient (0.165), even among the MZ pairs. This result indicate that environmental factors affect Ig-E level most among immunoglobulin levels.

IV-12. Genetic Analysis of Kawasaki Disease: **Fumiki HARADA, Takehiko SASAZUKI** (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **Masaharu SADA, Tetsuro KAMIYA** (Res. Inst. Dept. Pediatr., Natl. Cardiovasc. Cent., Suita), **Hideo MATSUMOTO** (Dept. Legal Med., Osaka Med. Coll., Takatsuki), **and Tomisaku KAWASAKI** (Dept. Pediatr., Jpn. Red Cross Med. Cent., Tokyo)

We examined HLA-A, B, C, DR and immunoglobulin allotype (Gm and Km) of 23 families with 63 members where two siblings were affected with Kawasaki disease (mucocutaneous lymphnode syndrome: MCLS). Analysis of 23 probands did not confirm the

statistical association of MCLS with HLA, Gm and Km. Out of 23 affected sib pairs, 4 shared two HLA haplotypes identical by discent, 12 shared one HLA haplotype and 7 shared no HLA haplotype. The distribution of HLA haplotypes shared by affected sib pairs did not significantly differ from the random distribution. Similarly, there was no distortion in the distribution of Gm haplotypes shared by the affected sib pairs. We sent questionnaires by mail to the pediatrician of 954 hospitals all over Japan, and obtained answers reporting 122 twin pairs from 575 hospitals (60.3%) by the end of June 1984. Out of 122 twin pairs, zygosities of 97 pairs had been estimated. The twin concordance was found in 17.5% of monozygous pairs and 10.0% of dizygous pairs, this difference being not statistically significant ($p=0.23$). All these results seem to indicate that genetic factors might not be involved in the pathogenesis of MCLS at all. However it still, of course, exists a possibility that genetic factors involved in the pathogenesis of MCLS might be masked by massive environmental factors during the epidemic period in Japan.

IV-13. Study of the Consanguinity in Isolated Community, Miyamacho (II) : Kazuo MANO, Masuji MORITA and Norio FUJIKI (Dept. Intern. Med., Fukui Med. Sch., Fukui)

We have made medical and genetic survey in two mountainous communities (A and K hamlets) in Miyamacho, Fukui Pref., where we have examined 516 members among 876 inhabitants for the genetic clarification of the rare inherited diseases and common adult diseases, and reported medical survey at the annual meeting of Japanese Society of Internal Medicine on April 1984. This time, we have reported the breeding structure of these two hamlets from the data basis transcribed into pedigree charts from the microfilmed Koseki files. It has revealed such high endogamy rate as 28.7%, and 32.5% in K hamlet (endogamy rate in the hamlet and that within Miyamacho) and 21.7% and 36.9% in A hamlet, which did not correlated with the geographical isolation and assumed as different historical backgrounds. The isolation breaking based on the transportation development has been observed as previously reported and as in other surveyed areas. However, the inbred rate, 1 C marriage rate and mean inbreeding coefficient of each hamlet was 24.3%, 6.5% and 0.00803 in K hamlet, and 21.0%, 16.1% and 0.01235 in A hamlet, respectively. High mean inbreeding coefficient still persists instead of decreasing 1 C marriage rate. There are in average slightly remote consanguinity of 2 C marriage, the same relationship from the common ancestor, great grand-parents, although these hamlets are not so distant from the Fukui city. Instead of isolate braking, there are still high consanguinity rate. The distribution of blood typing has revealed the different gene frequency deviated from general population of Fukui Pref., such as $I^A=0.262$, $I^B=0.218$, $G^M=0.620$ in K hamlet and 0.396, 0.112 and 0.612 in A hamlet.

IV-14. わが国における最近の近親婚率について：今泉洋子（厚生省人口問題研）：**Recent Survey of Consanguineous Marriages in Japan:** Y. IMAIZUMI (Inst. Population Problems, Minist. Health Welfare, Tokyo)

日本全国から 6 地区（旭川地方，多賀城市，身延町，岡崎市，川西市，福江市）を選んで，そこに在住している夫妻共に 65 歳未満の夫婦 9,225 組について，昨年の 9 月 1 日にアンケート法によって近親婚率を調べた．結果は次のとおりである．調査地区全体の近親婚の頻度をみると，いとこ婚率 1.58%，いとこ半婚率 0.55%，またいとこ婚率 1.00%，またいとこ半婚率 0.28%，その他の近親婚率 0.47%，全近親婚率 3.88% を得た．地域別にみた全近親婚率は 0.78%（旭川地方）から 7.89%（福江市）の間に分布していた．次に，結婚年次別に近親婚率をみると，近親婚率は結婚年次とともに減少しており，最近の 6 年間（1977～1983 年）に結婚した夫婦のいとこ婚率は 0.22%，全近親婚率は 0.96% であった．次に，近親結婚をした 358 組の夫婦に，なぜ近親婚をしたのかを尋ねたところ，「親などの勧め」と回答した者は 44%，「気心が知れている」と回答した者は夫が 30%，妻が 24%，「幼な友達」と回答した者は 5.3% であった．これらの理由を地域別にみると，旭川地方を除いたすべての地域において一番高い項目は「親などの勧め」，次が「気心が知れている」であった．なお，旭川地方では順位がこの逆であった．

IV-15. 羊水染色体検査における *de novo* 相互転座について：木下芳広（慶応健康相談センター），**田村昭蔵**（慶応大・医・産婦人），**栗原武久・鈴木健治**（警友病院・産婦人）．***De novo* Reciprocal Translocations at Amniocentesis:** Yoshihiro KINOSHITA (Keio Health Counseling Cent., Tokyo), Shozo TAMURA (Dept. Obst. Gynec., Keio Univ., Tokyo), Takehisa KURIHARA and Kenji SUZUKI (Dept. Obst. Gynec., Keiyu General Hosp., Yokohama)

われわれは，12 年間の羊水検査で 2 例の *de novo* 相互転座を経験した．1 例は 41 歳の高年妊娠からのもので，胎児核型は 46,XY,t(4; 9) (q35; q22) であったが，両親は正常核型であった．したがって，胎児は *de novo* 均衡型相互転座と判明した（羊水染色体検査では本邦初例と思われる）．他の 1 例は 36 歳の高年妊娠からのもので，羊水過少症のため総穿刺羊水量は 1.2 ml であったが Chang medium を用いて培養に成功し，complex chromosome aberration が認められた．即刻両親の検査を行い，母親の均衡型相互転座を確認してから，結局，胎児核型は 45,XX,t(1; 19) (p13; p13)mat, −19 pat, −22, +der(19 pat), t(19 pat; 22) (q13; q11) *de novo* であることがわかった．2 例とも両親の希望により妊娠中絶となり，前者からは胎児の外表異常は認められなかったが，後者からは多発奇型が認められた．

とくに第 1 の症例のような *de novo* 均衡転座は困難な問題を抱えている．それは *de novo* 均衡型 rearrangements は mental retardation の集団で多く見つかっており，その頻度は新生児集団の約 7 倍に達するといわれているからである．また，先天性形態異常の risk も高い（～14%）といわれる．しかし *de novo* 均衡型相互転座を持ちながらなんら異常が認められない者もいるわけで，これらの間の相違は明らかでない．この困難な問題の解決のためには，新生児染色体 screening からの *de novo* 均衡型 rearrangements の追跡調査等が必要である．

IV-16. 羊水検査の適応の年次推移について：木下芳広（慶応健康相談センター），**田村昭蔵**（慶応大・医・産婦人）．**An Annual Change of Indication for the Amniocentesis in Keio Group:** Yoshihiro KINOSHITA (Keio Health Counseling Cent., Tokyo) and Shozo TAMURA (Dept. Obst. Gynec., Keio Univ., Tokyo)

染色体検査を主としたわれわれの羊水検査実施件数は，1972 年から 1984 年 9 月末までに 767 件に達した．日本では欧米に比べて羊水検査の適応のうち高年妊娠 (advanced maternal age, 穿刺時満 35 歳以上，以下 AMA) の比率が小さいといわれていたが，当施設では年を追って AMA の比率が増大している．1972 年から 1978 年までの 7 年間は AMA の比率が 37.5% (192 件中，72 件)，1979 年から 1981 年までの 3 年間は 56.1% (221 件中，124 件)，その後の 1982 年から 1984 年 9 月末までは 68.7% (354 件中，243 件) であった．1983 年に羊水検査を行った 127 件でみると，35～39 歳が 68 件，40 歳以上が 28 件で，合わせて AMA の適応が 96 件 (75.6%) であった．これに対し前回染色体異常児 (previous child with chromosome aberration, 以下 PCA) の適応は 16 件 (12.6%) であった (いずれも他の適応の重複を含む)．このように，当施設では羊水検査適応の第 1 位はすでに AMA が占めており，第 2 位の PCA を大きく引き離し欧米型に近づきつつある．日本で AMA の比率が小さい原因は，胎児診断に対する社会的コンセンサスが十分得られていないとはいえ，産婦人科医の意識の差にもあると思われる．なお，1983 年の 127 件の羊水検査から 6 例の染色体異常と 2 例の AFP 異常高値 (1 例の染色体異常を含む) を認めているが，染色体異常の 4 例は AMA の適応であった．

IV-17. Y/21 転座染色体を保有する夫との間にできた胎児の出生前診断について：鈴森薫・今泉克英・久岡孝子・八神喜昭（名古屋市大・医・産婦人），**西山幸男**（三重大・医・産婦人）．**Prenatal Diagnosis in a Case of Paternal Translocation Carrier, 45,X, −Y, −21, +t(Y; 21):** K. SUZUMORI, K. IMAIZUMI, T. HISAOKA, Y. YAGAMI (Dept. Obst. Gynec., Nagoya City Univ., Aichi) and Y. NISHIYAMA (Dept. Obst. Gynec., Mie Univ., Mie)

Y 染色体と常染色体の転座はきわめてまれである．今回，私どもは，45, X, −Y, −21, +t(Y; 21)〔無蛍光 Y〕を保有する夫との間にできた妊娠において胎児染色体の出生前診断を行い興味ある結果を得た．この夫婦は結婚後 2 年経過するも子供が得られず，挙児希望にて諸検査を受けたところ，夫に無精子症？がみられ，その原因について染色体検査が行われたところ上記の結果が得られた．挙児は困難であろうとのことで AID を勧められていたところ，思いがけなく妊娠した．夫が転座保因者であるために胎児染色体の検査が必要と言われ，名古屋市立大学を紹介された．夫の転座染色体から派生する胎児染色体構成について解説し，妊娠 18 週に羊水穿刺を行った．羊水培養細胞による胎児染色体分析結果は 45,X,15p+ で，No. 15 染色体の短腕部に過剰部分が認められた．夫の Y 染色体が無蛍光のため Q- バンド法によってもこの過剰部分の同定が不可能であったが，夫の No. 21 染色体に付着した Y 染色体部分と酷似しているために，No. 15 染色体に Y 染色体が転座した可能性も考え，このまま妊娠を継続することにした．妊娠 23 週時の超音波画像診断により胎児性器は男性型であることから，t(Y; 15) であろうと推察された．妊娠経過は順調で，妊娠 37 週 2,950 g の正常男子が出産された．本症例では，No. 21 染色体に転座していた Y 染色体が，精子形成過程中においてであろうか，No. 15 染色体に移動したものと推定される，細胞遺伝学的に興味ある現象がみられた．

IV-18. 当科における出生前診断について：先天奇形の胎児診断例：飛弾修二・野原当・朴 魯慶・末原則幸・谷澤 修（阪大・医・産婦人）. **Four Cases of Congenital Anomalies Diagnosed Prenatally:** S. HIDA, A. NOHARA, R. PARK, N. SUEHARA and O. TANIZAWA (Dept. Obst. Gynec., Osaka Univ., Osaka)

当科では，昭和 50 年より遺伝相談，胎児診断外来を設け，遺伝性疾患，染色体異常に関する遺伝相談や出生前診断を行ってきた．すでに，遺伝性疾患や先天異常児の出産既応のある妊婦以外においても，妊娠中に羊水過多・過少を合併した例や，とくに訴えがなくても妊娠中に行われた超音波検査などにより，先天奇形が診断，あるいは疑われる例も少なくない．遺伝的な疾患，あるいは奇形症候群に関する出生前診断について検討した．症例 1：36 歳の HMG-HCG 療法，A.I.H. により妊娠した妊婦で，子宮内発育遅延 (I.U.G.R.)，羊水過多を伴った症例において，18-trisomy syndrome を診断した．症例 2：羊水過多，髄膜瘤が疑われた症例において，超音波検査および胎表造影により，臍帯ヘルニア，口蓋裂，過短臍帯，羊膜剥離等の所見を得，総合的に amniotic band syndrome と診断した．症例 3：双胎の 1 児に，羊水過少，胎児腹水，腎腫大を認めたが，他児には羊水造影，染色体検査にても異常を認めず，妊娠を継続し生児を得た．第 1 児は生後 2 時間 27 分で死亡したが，剖検にて infantile-polycystic kidney と診断された．症例 4：第 1 子，第 2 子が軟骨無発生症で，3 回目の妊娠時に，妊娠 22 週において超音波検査にて，achondrogenesis を診断した．

IV-19. Prenatal Diagnosis in Osaka University Hospital: Prenatal Diagnosis by Amniotic Cell Culture: Ataru NOHARA, Syuuji HIDA, Ro-Kung PARK, Noriyuki SUEHARA and Osamu TANIZAWA (Dept. Obst. Gynec., Osaka Univ., Osaka)

We have carried out genetic counseling and prenatal diagnosis in Osaka University Hospital since October, 1975. We have accumulated 225 cases prenatally diagnosed. Among 193 cases prenatally diagnosed by chromosome analysis, previous Down syndrome were 103 and parental translocation carriers were 17 cases. The prenatal diagnoses for metabolic diseases were done in 16 cases. All of the pregnancies with parental balanced translocation were checked by amniocenteses. One case of unbalanced translocation and 6 cases of balanced translocation carriers were diagnosed. One Down syndrome was diagnosed in 103 pregnancies with previous Down syndrome. Four cases of 21 trisomy, one case of 18 trisomy and one case of 13 trisomy were diagnosed in 37 pregnancies with advanced maternal age (over 35 years old). Two affected fetuses with metabolic disorders were diagnosed in 16 pregnancies. In the 17 pregnancies with obstetrical abnormalities such as hydroamnios and intrauterine fetal growth retardation (IUGR), 3 cases of chromosomal abnormalities were diagnosed, and all of them were 18 trisomy. The chromosome analysis is usefull not only for the cases of parental translocation, advanced maternal age and previous chromosomal abnormalities but also for the cases with obstetric complications such as hydroamnios and IUGR.

IV-20. A Family with Fanconi Anemia: Detection of Patients and Carriers Using Lymphoblastoid Cell Lines: Tomoko HASHIMOTO,[1] Takahiko SUKENAGA,[2] Mami YAMASAKI,[3] Keiichiro YOSHIOKA,[4] Yoshihiro YAMAMOTO,[1] Kazue NAKAMURA[1] and Jun-ichi FURUYAMA,[1] ([1]Dept. Genet., [2]Dept. Radiol., Hyogo Coll. Med., Nishinomiya; [3]Dept. Neurosurg., [4]Dept. Pediatr., Osaka Natl. Hosp., Osaka)

Fanconi amemia (FA) is one of the autosomal recessive diseases with high risk of cancer. Swift reported FA-carriers also had a high predisposition to cancer, and Auerbach *et al.* reported that the FA-carriers could be detected by induction *in vitro* of chromosomal aberrations using diepoxybutane (DEB). The onset of FA is variable even among members of the same family. Then it is important to diagnose FA-patients preclinically and to detect FA-carriers when a member of a family is diagnosed as FA. The proposita, FA1NI, was born in 1976 with an extra left thumb; the patient came to our hospital at the age of 5.8 years because of aplastic anemia. Chromosomal analysis from the peripheral blood lymphocytes revealed high frequency of chromosomal aberrations. Her parents were not related and her two sibs were healthy; spontaneous and DEB-induced chromosomal aberrations were the same as in the controls. For further experiments, lymphoblastoid cell lines (LCLs) were established. Survival cell counting after loading of mitomycin C (MMC) or DEB revealed no hypersensitivity in the LCLs from the FA family members except FA1NI-LCL. This indicated no other patients in the family. Though the induction of chromosomal aberrations by DEB (10^{-9} M, 72 hr) failed to detect the carriers, MMC (50 ng/ml, 72 hr) induced chromosomal aberrations in all LCLs from the FA family members at the intermediate level between those of FA-LCLs and control LCL. These results suggested that MMC could be used to detect FA carriers.

IV-21. 家族性球脊髄筋萎縮症(Kennedy-Alter-Sung 病)の一家系—罹患者および保因者の検討—：落合　淳・北本哲之・石本進士・大西晃生・後藤幾生・黒岩義五郎（九大・医・神内）. **A Family of Familial Bulbospinal Muscular Atrophy: A Study of Patients and Carrier**: J. OCHIAI, T. KITAMOTO, S. ISHIMOTO, A. OHNISHI, I. GOTO and Y. KUROIWA (Dept. Neurol., Neurol. Inst., Kyushu Univ., Fukuoka)

家族性球脊髄筋萎縮症(以下，本症と略)は，中年男性に緩徐に発症する神経原性筋萎縮症であり，伴性劣性遺伝を呈し，女性保因者の発見は臨床上，重要と考えられる．今回私たちは，本症の一家系について検討したので報告する．発端者は，49 歳男性で 30 歳頃より手のふるえに気付き，40 歳頃つま先立ちができなくなり，同じ頃より muscle cramp を認めた．神経学的には，舌および四肢に筋線維束挛縮，筋萎縮および脱力を認めた．心電図で心室内伝導障害を認めた．発端者の兄，69 歳男も，舌，四肢に筋線維束挛縮，筋萎縮および脱力を認め，心電図で I°A-V ブロックを認めた．保因者と考えられる発端者の 22 歳および 17 歳の娘は神経学的に muscle cramp 以外の異常を示さなかったが，心電図で心室内伝導障害を認めた．父，母方のいとこは，神経学的，心電図に異常を認めな

かった．本症は，高血圧，性腺機能低下などの随伴症状が知られている．しかし，保因者に関しての検討は少ない．本家系では，罹患者と保因者の共通の所見として muscle cramp と心電図変化が認められた．これらの所見は，保因者の検索上重要と考えられる．

IV-22. Birth Defects Monitoring in the Tokyo Metropolitan Hospitals: Kiyotaro KONDO, Kyoko KATO, Taeko AKITA and Isao HEMMI (Dept. Neurol., Tokyo Metropol. Inst. Neurosci., Tokyo)

All 55,103 neonates, including 808 still births after the 16th gestational week, born in 11 Tokyo Metropolitan hospitals during April, 1978–December, 1982, were monitored. Neonates with recognizable birth defects within 1 postnatal week were exhaustively collected. 4,006 neonates showed one or more of the defects, but major defects with functional impairments were observed in 698, or 1.3% of the neonates, being identical to the figures in other reports. Multiple defects totaled 464, including 54 chromosome anomalies and 30 genetic syndromes. Of 3,541 single defects, 3,126 were classifiable by the codes for the birth defects in the International Classification of Diseases. Temporal trends were evaluated with three methods in 17 selected defects including 11 designated by the Clearinghouse. The results were 1) a rise was alway transient and random being followed by a period with the base-line frequency, the overall trends being stationary, 2) a sharp rise of anencephalus in July, 1982 was due to four cases happended within several kilometers in downtown Tokyo around Koto-ku area, 3) polydactyly and syndactyly showed indentical results, 4) suitableness of three methods were compared with basically identical results.

IV-23. A Report on Osaka Birth Defects Monitoring Program (OBDMP): N. SUEHARA, K. KURACHI (Dept. Obst. Gynec., Osaka Univ., Osaka), **T. OURA, T. FUJINO** (Child. Med. Cent. Osaka City, Osaka), **T. TANIMURA** (Dept. Anat. Kinki Univ., Sayama-cho, Osaka), **J. LURUYAMA** (Dept. Genet., Hyogo Coll. Med., Nishinomiya), **M. Imagawa, S. TERAMURA** (Osaka Med. Assoc., Osaka), **M. FUKUI** (Osaka Soc. Obst. Gynec., Osaka), **T. TAKEMURA, A. HAYASHI, A. SASAKI, T. KAWAMURA** (Osaka Med. Cent. Res. Inst. Maternal Child Health, Izumi, Osaka) and **S. OGITA** (Osaka Municipal Maternity Infant Cent., Osaka)

The Osaka Birth Defects Monitoring Program has started at December 1981 as a population-based birth defects monitoring system in Osaka Prefecture. The 22 marker malformations and other major malformations have been monitored for all live births and stillbirths over 24 gestational weeks (and/or 500 g) within 7 days after birth. In the first 31 months, 156,253 records were collected from 285 obstetric clinics and hospitals in Osaka Prefecture and this corresponded to about 60% of all births in Osaka Prefecture at the same period. Overall incidence of total malformations (marker malformations, others and com-

bined) in all births was 1.06%. The incidence of anencephalus (for 10,000 births) was 7.3, that of spina bifida 3.7, that of hydrocephalus 3.6, that of cleft palate (without cleft lip) 5.1, that of cleft lip (with or without cleft palate) 13.3, and that of Down syndrome 5.9. The incidence of malformations in premature births was 3.54%, low birth weight (under 2,500 g) 4.06%, that of intrauterine fetal death 11.3%, and that of maternal diabetes mellitus 5.35%. About 95% of anencephalus and 67% of encephalocele had been diagnosed prenatally. The incidence of each marker malformation has been monitored every 3 months.

IV-24. Local Characteristics Noticed in Genetic Counseling in Fukui: Norio FUJIKI, Masuji MORITA and Kazuo MANO (Dept. Intern. Med., Fukui Med. Sch., Fukui)

We have performed genetic counseling at our university department and Fukui Health Authoritative for 2.5 years since Oct. 1981. One hundred thirteen cases were analyzed, comparing with those previously counseled in Kyoto (898 cases) and Nagoya (1,105 cases) areas. Significantly more persons came to the counseling through the local health authoritative in Fukui than in the other areas, while mass media has participated less, probably due to recent activities of public health nurses. The most common motive for conseling was the problem on the recurrent risk of the diseases at the time of marriage (46%) and of pregnancy (27%) in Fukui, as in other areas. The content of counseling was occupied by 1) herediatry disease (25%), 2) consanguinity (23%), 3) constitutional diseases including mental retardation and psychosis (21%), and 4) malformation (15%). Compared with other areas, herediatry disease and consanguinity were significantly more frequent problems and non-genetic disease was infrequent in Fukui. This finding suggests that genetic problems have still latently existed without reasonable solution, but common sense for hereditary disease has spreaded gradually in public. The results of the consensus survey in Fukui have been reported at the annual meeting of Japanese Society of Constitutional Medicine in Oct. 1984. The results indicate that they had sufficient interests in genetics but their information was not enough to banish the misunderstanding and prejudice against heredity and birth defects.

IV-25. 質問紙表による Flusher および Non-flusher の判別の試み：山田一朗・浅香昭雄（東大・医・保健），**竹下達也**（山梨医大・保健）. **Discrimination of Flusher and Non-flusher by Questionnare:** Kazuaki YAMADA, Akio ASAKA (Sch. Health Sci., Univ. Tokyo, Tokyo) and Tatsuya TAKESHITA (Dept. Health Sci., Med. Univ. Yamanashi, Yamanashi)

Low K_m アルデヒド脱水素酵素 (ALDH-I) の活性を欠くいわゆる flusher と，そうでない non-flusher との鑑別には，もっぱら毛根資料から等電点電気泳動による方法が用いられている．しかし，

対象者の数が大きい場合には，この方法は時間的にも費用の点でも問題がある．そこで今回，医学部の学生およびその知人を中心に男子 85 名，女子 43 名，計 128 名を対象として，自覚症状などからALDH-I の表現型を同定するための簡便な質問紙表の作成を試みた．質問紙表は，「顔面紅潮の有無」「かゆみ」「めまい」「眠け」「頭痛」「発汗」「吐き気」「心拍数増加」「寒気」「息苦しさ」など 13 の自覚症状について，「飲酒時はいつも出る」「時々出る」「全く出ない」の 3 段階で回答させる形式の項目を中心とし，そのほかに性別，年齢，飲酒期間，飲酒頻度等の質問を加えて作成した．これらの項目を説明変数とし，ALDH-J 表現型を外的基準として数量化II類による分析を行った．各カテゴリーに与えられた重み係数の総和が負の値をとるとき ALDH-I 欠損型 (flusher)，正の値のとき ALDH-I 非欠損型 (non-flusher) を示すように設定して計算した．求められた重み係数の数値を用いて，質問紙表からの判定と実際の表現型がどの程度一致するかを調べたところ，全体としての的中率は 83.5% となった．

IV-26. Genetic Background of "Shyo" in the Treatment in Oriental Medicine: Takasi KANAYA,[1] Masatosi ABE,[1] Yukiko MARUYAMA,[1] Mizue MORITA,[2] Katuhiko INAGAKI[2] and Zen-ichi OGITA[1] ([1]Dept. Pathol. Biochem., Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama; [2]Inagaki Clinic, Toyama)

In oriental medicine, treatment is performed on the basis of "Shyo." In the treatment based on "Shyo," patient's constitution or symptom is classified according to two opposed "Shyo," *e.g.* "Hyo-Ri," "Netu-Kan," "Jitsu-Kyo," "Sou-Sitsu" and others, and the treatment method is decided. However, no one has verified the existance of "Shyo" or its relating genetic background. In the case of rheumatism, the patients have been classified as "Kan-Kyo-Sitsu Shyo," because of its consumptive nature. In the present study, the distribution of "Shyo" was analyzed by making a statistic and objective comparison between rheumatic patients and healthy subjects. Such analysis, we believe, will clarify the existence of "Shyo" and explicate its genetic background. The general questionnaire concerning oriental medicine was handed out to the two groups of subjects, 301 rheumatic and 281 healthy subjects, and each person described one's own subjective symptoms according to the questionnaire. The each answer was scored according to the basic classification of oriental medicine—"Netu-Kan"–"Jitsu-Kyo"–"Sou-Sitsu"—score sheet and "Shyo" was determined. We also analyzed pedigrees which contain the patient. In the results, the existence of "Shyo" associated with rheumatism was verified. Also, we discussed the possibility of the existence of genetic background for "Shyo" from the results of pedigree analysis.