日本人類遺伝学会第 27 回大会講演要旨 (1982)

Abstracts of Papers Communicated at the 27th Annual Meeting of
the Japan Society of Human Genetics, 1982

一般講演 General Contribution

- A1. The Classification and Counting up of Congenital Anomalies: Kazumi NAITO**
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It is the base of medical and social measures against congenital anomalies to know the actual circumstances of their occurrences. As one step of searching for the ways to know the actual occurrences of congenital anomalies, we have tried to make and applied practically a list of congenital anomalies. The list was made by picking up congenital anomalies out of the all items of The International Classification of Diseases (I.C.D.) with reference to "Nelson Pediatrics." Consequently, 312 items including 190 ones belonged to the Chapter XIV of I.C.D., "Congenital Anomalies" were listed up. And further, 131 congenital anomalies classified together with acquired diseases in the other items were confirmed.

Secondly, we tried to apply only the list of 312 items of congenital anomalies to the statistics of diseases on 4,347 children who have admitted to Kanagawa Children's Medical Center in the fiscal year of 1980. The number of inpatients whose primary reason for admitting were diseases belonged to the Chapter XIV, mainly included congenital malformations, were 1,286 (29.58%). They were 426 inpatients whose main diseases belonged to "Anomalies of heart and circulatory system," 233 of "Anomalies of genito urinary system," 168 of "Anomalies of musculoskeletal system" and so on. And 1,825 inpatients (41.98%), including 1,286 ones above mentioned, were counted up according to our list. As congenital anomalies in the other chapters, 205 of "Congenital strabismus," 203 of "Inguinal hernia," 32 of "Coagulation defects," 23 of "Haemangioma and lymphangioma," 16 of "Haemolytic disease of fetus, according to isoimmunization," 11 of "Congenital hypothyroidism," and so on were found. The necessity of intentional devices to grasp the actual occurrences of congenital anomalies, and the gravity of them in the medicine for children of high level were shown.

- A2. 久留米市における、精神発達遅滞の年度別有病率と成因について：塩月由子・松石豊次郎・山下文雄（久留米大・医・小児）、吉村皓子（久留米保健所）、矢野和男・時政 博（久留米幼児教育研）. Prevalence Rate and Etiology of Mental Retardation in the Different Year in Kurume City: Y. SHIOTSUKI, T. MATSUIISHI, F. YAMASHITA (Dept. Pediat., Kurume Univ., Kurume), K. YOSHIMURA (Kurume Public Health Cent., Kurume), K. YANO and H. TOKIMASA (Kurume Infant Edu. Cent., Kurume)**

久留米市（人口 21 万人）は昭和 56 年度に心身障害児早期療育研究会を結成し、心身障害児の発生子防と早期発見早期療育のシステム確立のため障害児の実態調査を行った。昭和 44 年度から昭和 49 年度までに出生した市内在籍児 21,622 人を対象とした。久留米市心身障害児早期療育研究会は医療教育福祉の各関係機関のメンバーより構成され、情報をもちより一本化して行った。調査は精神発達遅滞児の出生年度別、性別、程度別、成因別に分類し、程度判定はウィスク法、ビネー法などを用い、IQ 70 以下を精神発達遅滞とした。有病率は、その年度の在籍児数で各年度の精神発達遅滞児数を除して人口千対比であらわした。

結果は、1) 久留米市における精神発達遅滞の平均有病率は人口千対 7.1 であった。2) 精神発達遅滞の有病率は 6 年間に於いて増減がなく一定していた。3) 性別では男児が女児の約 2 倍高い有病率であった。4) 成因別では明らかな出生後要因は 14% と少なく、成因不明、出生前要因などの不明な要因が多かった。

- A3. 脳回転状網脈絡膜萎縮 (Gyrate atrophy of the choroid and retina) の遺伝子頻度および有病数の推定：藤木慶子・早川むつ子・中島 章（順天堂大・眼科）. Estimation of the Gene Frequency and the Prevalence of Gyrate Atrophy of the Choroid and Retina in Japan: K. FUJIKI, M. HAYAKAWA and A. NAKAJIMA (Dept. Ophthalm., Juntendo Univ., Tokyo)**

脳回転状網脈絡膜萎縮は視力障害と夜盲を訴え、高度近視を合併し、進行するにつれて視野は狭窄し、暗順応や網膜電図も異常となり、あたかも脳回転を思わせる特異的な眼底となる稀な常染色体性劣性遺伝様式をとる疾患である。近年、本症のほとんどの例に高オルニチン血症を伴うことが確認され、ornithine ketoacid transaminase (OKT) の欠損ないしは活性の低下に基づくことが明らかになった。その特異な眼底所見にもかかわらず、演者らの検索において本症と確認しえた本邦での報告例は河本 (1913) 以来現在まで 13 家系 17 症例にすぎない。また、北川ら (1982) の新生児 8 万人に対するスクリーニングにおいて 1 例も発見されなかった。13 家系中近親婚が 9 組 (69%) に及ぶことからきわめて稀な疾患であることがうかがえるが、本症の臨床症状が網膜色素変性症とまぎらわしいため、実際はもっと存在すると推定される。そこで、13 家系中いとこ婚とわかっている 6 組、 $k=0.4615$ 、いとこ婚か否か不明の 2 組のうち 1 組がいとこ婚としたときの $k=0.5385$ 、2 組がともにいとこ婚としたときの $k=0.6154$ と本症両親の推定結婚年から求めた一般集団中のいとこ婚率 $c=0.0375$ から本症の劣性遺伝子頻度を $0.0016 \sim 0.0030$ 、発症頻度 $(3.6 \sim 11.0) \times 10^{-6}$ 、 $(9 \sim 28)$ 万人に 1 人、有病数を 400～1,300 人と算出した。

以上の結果は少ない症例からの推定であるので幅をもって推測したが、あくまでスクリーニングおよび患者発見の目安とし、今後の症例の出現を待って検討していく所存である。

A4. 某病院における外表奇形 34 年間の統計的分析：今泉洋子（厚生省人口研），山村博三・西川政子（聖バルナバ病院）. A Statistical Analysis of Birth Defects in Newborns, 1948-1981: Y. IMAIZUMI (Inst. Pop. Prob., Tokyo), H. YAMAMURA and M. NISHIKAWA (St. Barnabas' Hosp., Osaka)

1948 年から 1981 年までの 34 年間に某病院で出産した 110,785 名を対象に，出産後 1 週間以内に診断された外表奇形を調べた。

奇形の内訳は多指症が一番多く 90 人，無脳症 75 人，口唇裂 66 人，口蓋裂を伴った口唇裂 65 人，合趾症 63 人，口蓋裂 55 人，多趾症 47 人，鎖肛 31 人，二分脊椎 27 人，先天性水頭症 24 人，臍ヘルニア 22 人，合指症 21 人であった。一方，単一遺伝子座によって決まる遺伝病としては Achondroplasia 9 人，真性小頭症 5 人，アペルト症候群，裂手・裂足，先天性魚鱗せん，ルロイ症候群，エリスーバン・クレペルト症候群，第Ⅻ因子欠乏症がおのおの 1 人ずつ見つかった。

次に，奇形発生率の動向を見るため，古い年次群（1948～1968 年）と新しい年次群（1969～1981 年）とに分け発生率の比較を行った。その結果，大部分の奇形の発生率には変化が見られなかったが，口蓋裂を伴った口唇裂，尿導下裂，臍ヘルニア，臍帯ヘルニアでは，新しい年次群のほうが統計的に有意に高い発生率が得られた。なぜ，これらの奇形の発生率が上昇したのかは，今後の結果とあわせて検討したい。

A5. A Preliminary Report on Kanagawa Birth Defects Monitoring Program (KAMP): Yoshikazu KUROKI, Hiroshi KONISHI, Minoru MAEDA, Akio TSUNODA, Yoshiaki TERAMICHI (Kanagawa Child. Med. Cent., Yokohama), Fumio TADOKORO, Kenji ADACHI and Shinichi YAGI (Kanagawa Soc. Obstet. Gynecol., Yokohama)

There were two types of monitoring systems, namely, population-based and hospital-based. Though the Tokyo Area Birth Defects Monitoring Program (hospital-based) has participated in the activities of the International Clearinghouse for Birth Defects Monitoring Systems, no population-based monitoring systems exist in Japan prior to 1981. Kanagawa Birth Defects Monitoring Program has been in operation since October 1981 as the first population-based monitoring systems in Japan. In this program, 48 marker malformations and other major surface anomalies are monitored for all births (live births and stillbirths) within 7 days after birth. The program covers about 45,000 through 50,000 births per year in Kanagawa Prefecture. The overall frequency of the individuals with major surface anomalies was 1.2%. Stillbirth, low birth weight and small for dates are proved to increase the incidence of congenital malformations. A guide-book distributed to all participating maternity hospitals plays an extremely important role in increasing the completeness of ascertainment of malformations and in keeping the accuracy of diagnosis.

A6. 先天異常の全容把握と地域管理のモデル：松井一郎（愛知コロニー発達障害研）.

A Model for Registry of Birth Defects: Ichiro MATSUI (Aichi Pref. Colony, Kasugai)

神奈川県逗子市（人口 6 万，年間出生 700）で 1974 年より地域内全出生の先天異常・遺伝性疾患の把握を行った。従来の母子保健システムを改変し，先天異常の地域管理モデル（母子保健ケア・システム）とした。疾病の情報源は，母子手帳交付時・出生届時のインタビューからはじまり，3 カ月，6 カ月，1 歳，1 歳 6 カ月，3 歳の乳幼児健診，健診を受けないものの保健婦訪問，養育医療・育成医療・小児入院医療費援護の申請書，国保レセプト，医師からの連絡，医療相談その他。

活動の主体は保健婦で，上記の情報が対象児ひとりずつ一貫管理されている。先天異常の疑いのあるものは，ハイリスク児として登録されコンピュータに入力，診断確定，医療や療育の方向づけが行われる。診断レベルは乳幼児健診での再度の確認，近隣の病院小児科，大学病院，こども医療センターなど。

1 歳 6 カ月をこえた対象児 4,602 名（1975～1980 年生まれ）の集計（転入・転出除外）ではハイリスクレジストリーが 25% に達した。精密検査確定および手術等の治療を行った先天異常は 5.7% であった。

A7. 先天性代謝異常症監視の統計的方法：安田徳一（放医研・遺伝）. Statistical Methods in a Surveillance System of Congenital Abnormalities, with a Special Reference to Inherited Metabolic Diseases in Japan. Norikazu YASUDA (Div. Genet. Natl. Inst. Radiol. Sci., Chiba)

先天異常の発生数を時間的に監視していくと，発生数にゆらぎが見られる。先天異常監視の基本の一つとして，このゆらぎを統計的にモニターすることが重要である。一般に先天異常の特徴として，個々の発生頻度（ p ）は低く（ $1/1,000$ 以下），対象集団の個体数（ N ）が多いことがあげられるが，これは統計学的に「発生数の分布は平均 $m(=Np)$ のポアソン分布に従う」と表現できよう。そこで，先天異常発生数の統計的監視方法としての Z 法，累積和法，逐次検定法にポアソン分布の性質を適用，開発し，昭和 52 年 4 月より日本全国で施行されている先天性代謝異常症の月別発生数に応用，検討した。

その結果，1) Z 法は一時点における急激な変化を，2) 累積和法は漸進的な変化を勾配として視覚にとらえるのに適している。3) 逐次検定法は 1)，2) の特徴を兼ね備えた方法で，対象となる先天異常の発生数に柔軟に対応が行えと考えられる。4) 検査率がほぼ一定となった昭和 54 年以降の 27 カ月間で，先天性代謝異常症総数 595 名，検査実施総人員数 3,204,778 名。したがって日本のペースラインは 100 万出生あたり 186 名となる。先天性代謝異常症別ではフェニルケトン尿症 18 名，楓糖尿症 2 名，ヒスチジン血症 142 名，ホモシスチン尿症 6 名，ガラクトース血症 11 名，その他 7 名となる。5) これら先天性代謝異常症はいずれも単純劣性遺伝形質なので，発生頻度が急に変化するとは考え難く，むしろヒトあるいはシステム側に問題があると考えられる。今後，先天異常をモニタリングするにあたり，その監視方法をチェックする上で発生頻度に大きな変化が起こらない疾患も含めて行う必要があると考えられる。

資料の提供をしていただいた厚生省母子衛生課前課長福渡靖，谷修一両氏に感謝します。

- A8. Study of Genetic Effects of Occupational Exposure to Mustard Gas (1):** Mikio FUJITA, Kazuaki GORIKI, Chiyoko SATOH, Howard B. HAMILTON (RERF, Hiroshima), Michio YAMAKIDO, Tsutomu INAMIZU, Kiyoshi ONARI, Yukio NISHIMOTO (Dept. Intern. Med., Hiroshima Univ.) and Takuzo SHIGENOBU (Health Cent., Hiroshima Univ., Hiroshima)

It is well known that former workers of the Ohkuno-jima poison gas factory located in Takehara City in Hiroshima Prefecture were exposed to mutagens such as sulphur mustard gas of relatively high concentration. Cancer prevalence among these people is significantly elevated. We are at present studying the genetic effects of exposure to this gas at the protein level, among the children of these individuals. This report describes the results of our search for rare variants conducted mainly by use of starch gel electrophoresis of peripheral blood samples obtained from 456 children born during employment and after termination of these former workers. In examining 5 serum protein systems, HbA₁ and HbA₂, one variant of α_1 -AT with slow migration rate, one variant of α_1 -AT with fast migration rate, 5 variants of Tf with fast migration rate, 3 variants of Hp with fast migration rate and one variant of Hp with slow migration rate were detected. Of the 23 types of erythrocyte enzymes, the following rare variants were detected: In the Np system 3 cases, PGM₁ 2 cases, PGM₂ 2 cases, GPI 3 cases, GPT 12 cases, GOT 2 cases and CAI one case. GPI one case and PGM₂ 2 cases are rare variants which have not yet been detected in the RERF "F₁ Genetics Study" sample. Family study has been completed on 32 cases, all of which were confirmed to have been inherited from one parent. Frequency of the rare variants was found to be 2.6/1,000.

- A9. Probability Distribution of Coefficient of Consanguinity in an Isolate Population:**
A. KUDÔ (Dept. Math., Kyushu Univ., Fukuoka), S. AZUMA, K. HAYASHI (Dept. Applied Math., Tokyo Sci. Univ., Tokyo), N. FUJIKI (Intern. Med., Fukui Med. Sch.), I. NISHIGAKI (Aichi Pref. Colony, Kasugai) and K. MANO (Fukui Med. Sch., Fukui)

This paper is motivated by a question, "Are people in an isolate population marrying randomly?" From a pedigree of an isolate population in Japan, we selected those who are at age 20-29 at the time of survey. There are 52 families with 135 children. We examined the relation between the inbreeding coefficient and sibship size, and there was found no correlation. The average coefficient of consanguinity of 52 parents are nearly equal to the average inbreeding coefficient of 135 children, which are about 0.0195.

We then computed coefficients of consanguinity for all possible $52 \times 52 = 2,704$ pairs and the average is 0.00793. In order to test the difference between the actual value 0.0195 and the expected value 0.00793, a certain mathematical theory was developed. There are $52! = 8.07 \times 10^{67}$ possibilities when the 52 males are to marry to those 52 females, and

equal probabilities are assigned to these possibilities, and we computed an approximate upper tail probability for the value 0.0195, which is about 0.0078, showing significance. This indicates that people did not marry randomly in the above sense.

Generally it is believed there have been some socio-economic factors in Japanese society which makes consanguineous marriages more frequent than in other countries. The above analysis indicates that the same factors were valid in this isolate population.

A10. Rare Variants of Erythrocyte Enzymes in Japanese of Hiroshima and Nagasaki Detected by Starch Gel Electrophoresis. I. Phosphoglucomutase₁ (PGM₁): Junko KANEKO, Naomi MASUNARI, Yasukazu KIMURA, Mikio FUJITA, Kazuaki GORIKI, Takeshi KAGEOKA, Norio TAKAHASHI, Chiyoko SATOH (RERF, Hiroshima), Ryuji HAZAMA, Shotaro NERIISHI and Sadahisa KAWAMOTO (RERF, Nagasaki)

Starch gel electrophoretic study of the erythrocyte enzymes of children of proximally and distally exposed parents in Hiroshima and Nagasaki has been performed, to evaluate the potential genetic effects of atomic bomb radiation. This report describes hereditary electrophoretic variants of PGM₁ encountered during this screening. No mutant of PGM₁ has thus far been encountered. The total number of examined children is 14,575, but is 10,484 when "representative children" who were the first among siblings to undergo the examination were selected from each sibship. Eleven kinds of rare electrophoretic variants detected by starch gel electrophoresis employing TEMM buffer, pH 7.4, of Spencer *et al.* were named following our rule of nomenclature of variants and they are described as follows in the order of their mobility, a, b, c, d-bands as well as main band of 7 being described as standards: d, 3_{NGS} 1(31), 5_{HIR} 1(1), c, 7, b, 4_{NGS} 1(1), 4_{HIR} 2(1), a, 6_{NGS} 1(1), 6_{NGS} 3(1), 6_{NGS} 2(6), 6_{HIR} 1(1), 6_{HIR} 3(3), 6_{HIR} 2(2), 8_{NGS} 1(7). Numbers in parentheses are the number of cases of variants detected in the "representative children." These variants were encountered as a heterozygous phenotype, *i.e.*, they were detected in combination with one of the three polymorphic allozymes of 1, 2, and 7, except for 4_{HIR} 2 which was found as homozygous phenotype. Six out of eleven variants had been detected in the previously reported population of atomic bomb survivors. Five variants of 5_{HIR} 1, 4_{NGS} 1, 4_{HIR} 2, 6_{NGS} 3, 6_{HIR} 3 have been detected for the first time in this population. Significant differences were observed in the two cities in the frequencies of PGM₁³NGS 1 and the total rare variants.

**A11. Rare Variants of Erythrocyte Enzymes in Japanese of Hiroshima and Nagasaki
Detected by Starch Gel Electrophoresis. II. Phosphoglucomutase₂ (PGM₂):
Naomi MASUNARI, Junko KANEKO, Yasukazu KIMURA, Kazuaki GORIKI,
Jun-ichi ASAKAWA, Norio TAKAHASHI, Chiyoko SATOH (RERF, Hiro-
shima), Ryuji HAZAMA, Kyoko TOYAMA (RERF, Nagasaki) and Keiko IWA-
MOTO (2nd Dept. Clin. Med., Hiroshima Univ., Hiroshima)**

Starch gel electrophoretic study on the erythrocyte enzymes of children of individuals proximally and distally exposed to the atomic bombs in Hiroshima and Nagasaki has been in progress. Whenever a rare electrophoretic variant was encountered in a child, the parents of the child was examined for the same variant to find out whether the variant has a hereditary origin or a mutational origin in the preceding generation.

A total of 14,588 children including about 28% of siblings of which 8,391 and 6,197 belonged to proximally and distally exposed group, respectively, were examined on PGM₂ by starch gel electrophoresis employing TEMM buffer, pH 7.4, of Spencer *et al.* Five kinds of variant phenotypes were detected in 11 children who showed sets of variant isozymes in combination with e, f, g-bands of PGM₂ 1. We named three fast-moving variants as 5_{NGS} 1 (1), 5_{NGS} 2 (1) and 2_{HIR} 1 (1), and two slow-moving variants as 9_{NGS} 1 (6) and 9_{NGS} 2 (1), digits in parentheses being number of the variants which exclude one case of 9_{NGS} 1 encountered in a brother of the proband. Difference in electrophoretic mobility between 5_{NGS} 1 and 5_{NGS} 2 is subtle but these two variants were clearly separated by isoelectric focusing. All these variants showed phosphopentomutase activity which is characteristic to enzymes produced by the alleles of PGM₂ locus. Family study for these variants confirmed that all of them are genetic variant except for one case of 9_{NGS} 2. Parents of the proband having 9_{NGS} 2 who were in the proximally exposed group of Nagasaki showed normal PGM₂ 1 and no variant isozyme was detected. As there were no discrepancy between stated and biological parentage on the basis of serological studies on blood typing, electrophoretic phenotypes of blood proteins and HLA phenotypes, 9_{NGS} 2 seemed to be a mutant reflecting the gene mutation.

**A12. Rare Variants of Erythrocyte Enzymes in Japanese of Hiroshima and Nagasaki
Detected by Starch Gel Electrophoresis. III. Triosephosphate Isomerase (TPI):
Jun-ichi ASAKAWA, Junko KANEKO, Kazuaki GORIKI, Chiyoko SATOH
(RERF, Hiroshima) and Ryuji HAZAMA (RERF, Nagasaki)**

Four TPI variants were identified by starch gel electrophoresis using TEMM buffer, pH 7.4 among 14,014 children comprising the same population described in Part I of this series, number of the children being 10,070 when siblings were excluded from the total. Each isozyme band of them was purified by means of polyacrylamide gel electrophoresis after phosphocellulose chromatography, ammonium sulfate fractionation and gel filtration.

These isozymes were compared with normal isozyme regarding their stability, kinetic parameters and immunological properties. Each band of a set of bands of the TPI 2_{HIR 1} variant migrated anodal to those of the normal type (TPI 1) in starch gel electrophoresis. This variant was less stable than normal in the guanidine denaturation test, and inactivation was only 65% of that of normal in a titration test using rabbit serum anti-human TPI. The level of activity in a hemolysate of a heterozygote for TPI 1-2_{HIR 1} was about 60% of normal. TPI 2_{NGS 1} is a variant which migrates anodally. In the heat denaturation test the normal isozyme required 52 and 25 min to lose 50% activity at 55°C and 57°C, while this variant required 26 and 13 min, respectively, an indication of instability of this variant. Moreover, this variant is also more labile than the normal type in stability tests in buffers at pH 5 and pH 10. TPI 4_{NGS 1} migrated cathodally and was more stable than the normal isozyme in pH 5 buffer. Biochemical and immunological characteristics of TPI 3_{HIR 1} which migrates anodally has already been reported¹⁾. Family studies for these four variants demonstrated that the unique characteristics of these variants are genetically determined.

Reference: 1) 1982. *Biochem. Genet.* 20: 59-76.

A13. The Frequency among Japanese of Heterozygotes for Deficiency Variants of 11 Enzymes: Chiyoko SATOH, Akiko MIURA, Chiemi UENO, Hiroko ARAKAWA, Hideo OMINE, Kazuaki GORIKI, Mikio FUJITA, Howard B. HAMILTON (RERF, Hiroshima) and James V. NEEL (Univ. Michigan, Ann Arbor)

Eleven human enzymes, chosen for this study because of relatively small coefficients of variation for mean activity, have been surveyed for the frequency of activity determinations $\leq 66\%$ of the mean value, for a total of 26,634 determinations. This criterion should detect almost all heterozygotes for variants lacking any activity plus a fraction of the persons with variants characterized by markedly depressed activity and/or instability. The enzymes surveyed are TPI, PGK, AK₁, LDH, GAPD, GPI, PK, 6PGD, G6PD, GOT₁ and HK. The number of determinations per enzyme ranged from 310 to 3,173. Family studies have thus far been possible in 52 instances in which the initial observation of activity $\leq 66\%$ of normal was confirmed. In every instance, a parent exhibited a similar finding, giving confidence a true genetic entity was being detected. The frequency of variants detected by this approach per 1,000 determinations varied from 0.0 (AK₁, 6PGD) to 13.8 (PK), with an average of 2.4. For these same systems in this laboratory the frequency of "rare" electrophoretic variants is 2.3/1,000, the ratio of the latter to the former thus being 1.0 in Japanese. Our experience with these deficiency phenotypes to date suggests that for selected enzymes they can be incorporated into a program designed to detect mutational events.

A14. Human Erythrocyte Carbonic Anhydrase Deficiency in Japanese Populations (Hiroshima, Nagasaki): Kazuaki GORI KI, Junko NISHIZAKI, Satomichi KANE-OKA, Eiko NISHIKORI, Ryuji HAZAMA (RERF, Hiroshima, Nagasaki) and Michio YAMAKIDO (2nd Dept. Intern. Med., Hiroshima Univ., Hiroshima)

Two isozymes of carbonic anhydrase, CA I and CA II are present in human erythrocytes. Hereditary CA I deficiency has been reported.¹⁾ We report here the results obtained from a quantitative survey of CA I and CA II employing single radial immunodiffusion. Blood samples were obtained from 3,160 offspring of atomic bomb survivors living in Hiroshima and Nagasaki. The enzyme concentration (mg) was expressed in terms of mg per g hemoglobin (g/Hb). Starch gel electrophoresis was carried out at the same time. The mean CA I concentration among 3,160 individuals was 12.4 mg/gHb (SD=2.2), and for 22 cases it was less than about 3SD of the mean. The mean concentration of these deficient cases was 5.5 ± 0.6 mg/gHb (mean $\pm$ SD). In 13 of 15 cases for which family study could be made, one of the parents showed low concentration of CA I as that of the proband. The 16 cases of 22 deficient cases showed normal esterase activity and normal thermostability. The frequency of CA I deficiency was 7.0 per 1,000 determinations, which was higher than the frequency of 0.7/1,000 of electrophoretic variants. The mean CA II concentration for the same population was 2.11 mg/gHb (SD=0.33), and for 2 cases showed a value less than 3SD of the mean. Family study for one of these two cases confirmed that the deficiency is genetic origin. Electrophoretic pattern was normal for all these deficient cases of CA I and CA II, though intensity of their bands were weak compared with that of normal bands.

Reference: 1) Kendall, A.G., and Tashian, R.E. 1977. *Science* 197: 471.

A15. Prevalence and Enzymological Characteristics of Electrophoretic Glucose-6-Phosphate Dehydrogenase Variants in Hiroshima and Nagasaki: Takeshi KAGEOKA (Univ. Tsukuba, Ibaraki), Kazuaki GORI KI, Mikio FUJITA, Chiyoko SATOH, Kaoru YAMAMURA, Junko KANEKO, Kenichiro ISHII, Naomi MASUNARI (RERF, Hiroshima)

A program of electrophoretic screening of glucose-6-phosphate dehydrogenase (G6PD) was carried out in Hiroshima and Nagasaki cities in Japan on blood samples from children born to proximally exposed survivors and their controls (children born to distally exposed survivors). The family studies were performed for the variants, and the characterization of partially purified G6PD preparations in the hemizygous males in the family was carried out by following the method recommended from WHO Scientific Group.

The prevalence of electrophoretic variants in Hiroshima was found to be 0.08% in males and 0.33% in females, while in Nagasaki it was 0.16% in males and 0.22% in females. In

the enzymological characteristics of partially purified G6PD preparations obtained from individuals hemizygous for electrophoretic variants, 3 new types of G6PD variants were identified among 5 variants detected in Hiroshima, and 4 new types of G6PD variants among 5 variants detected in Nagasaki. The individuals hemizygous for these variants all were classified as either class 3 or class 4, and the results of routine hematological tests were within normal limits and no hemolytic episode.

A16. Activity Variant of Human Erythrocyte Lactate Dehydrogenase (LDH): Junko NISHIZAKI, Kazuaki GORIKI, Chiyoko SATOH, Akiko MIURA, Chiemi UENO, Hiroko ARAKAWA and Hideo OHMINE (RERF, Hiroshima)

We are at present engaged in a screening program using enzyme activity as marker. As 80% of LDH activity in erythrocytes originates from the B sub-unit, it is difficult with a screening test using hemolysates as specimen to detect activity variants of the A sub-unit. Accordingly, by combined use of activity determination and agarose gel electrophoresis, the staining level of each isozyme fraction was determined, and the results obtained are reported. Blood samples were collected using ACD as anticoagulant from 1,029 children born to A-bomb survivors residing in Hiroshima. After erythrocytes were isolated and washed, 1/2 hemolysates were prepared using a β -mercaptoethanol-EDTA solution, and LDH activity was determined according to the method of ICSH (1977). Further, agarose gel electrophoresis was conducted on the hemolysates using tris-citrate buffer solution, pH 7.2. After formazan staining, densitometry was performed. Mean activity for 1,029 subjects was 159.65 IU/gHb (SD=14.4), and the ratios (%) of each isozyme fraction against all fractions were LDH 1: 35.6 (SD=2.1), LDH 2: 39.8 (SD=1.6), LDH 3: 19.9 (SD=1.8) and LDH 4: 4.7 (SD=1.3). For one LDH 1 fraction and for two other LDH 4 fractions, the mean activity was less than 3 SD of the mean for the corresponding normal fraction ratio. In a family study of the case with decreased LDH 1 activity, although total activity was normal for both the propositus and mother with 191.90 and 189.77 IU/gHb, each showed a significant decrease to 26.1% and 28.4%. These LDH 1 fractions were purified, then the thermostability and K_m for pyruvate were determined. The results were not different from the normal controls. In a family study of one of the cases with decreased LDH 4 activity, although total activity was normal for both the propositus and the mother with 135.16 and 143.43 IU/gHb respectively, for the LDH 4 isozyme fraction, each showed a significant decrease to 0.6%, thus indicating genetic transmission of this decreased activity variant.

- A17. Incidence of Red Cell NADH Diaphorase Variants in Japanese: K. SHIMIZU, H. KEINO** (Dept. Morphol., Inst. Develop. Res., Aichi Pref. Colony), **T. ITOH and I. NISHIGAKI** (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Human red cell NADH diaphorase isozyme patterns have been examined in 5,046 healthy and unrelated Japanese individuals by starch gel electrophoresis. 23 people with variant isozyme patterns were encountered: 20 were phenotype Dia 2-1 and only 3 were Dia 4-1. No other variant isozyme patterns were identified. We also have noticed that thin-layer isoelectric focusing on polyacrylamide gel (Pagif) is very useful for discrimination of true variant isozyme patterns from "aging bands" or "pseudopolymorphism." The frequency of the *Dia*² gene in the present study is almost same as that in European populations while the *Dia*⁴ gene frequency is extremely low in comparison with other populations. NADH diaphorase isozyme patterns have been also examined in patients mentally handicapped. The *Dia*² gene frequency in patients with mental retardation (313) and those with epilepsy (218) is significantly high although the tested number is very small. The enzyme activity of the newborn is almost half of that in the adult and is inversely proportional to the concentration of fetal Hb (Hb F) present.

- A18. Quantitative Variations in Polymorphic Types of Human Red Cell Esterase D: Itsuro NISHIGAKI, Tohru ITOH** (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony) **and Nobuaki OGASAWARA** (Dept. Biochem., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

Human red cell esterase D exhibits genetic polymorphism with three common phenotypes, EsD 1, EsD 2-1 and EsD 2, determined by two autosomal codominant alleles, *EsD*¹ and *EsD*². A large number of population data on allele frequencies of esterase D have been published, and hitherto the frequency of *EsD*² has been found to be lower in Caucasian and Negro populations, but higher in Mongolian races. This emphasizes that any Mongoloid population might be of better applicability for evaluating the quantitative characters in different phenotypes of esterase D. While, from the expression of isoenzymes of the heterozygote EsD 2-1, it is suspected that there might be some differences in activity between *EsD*¹ and *EsD*² gene products. To confirm this possibility, a quantitative analysis was performed on 336 Japanese samples. Using 4-methylumbelliferyl acetate as substrate, assay was carried out in a pH 5.2 solution. Among the three different phenotypes there was a marked variability in activity, namely the highest and lowest values were found in EsD 1 and EsD 2, respectively and that of EsD 2-1 was fallen between these two levels. Their mean values were as follows: 5.37 ± 0.42 units/gHb for EsD 1, 3.86 ± 0.32 for EsD 2-1 and 2.28 ± 0.17 for EsD 2, with a mean of 4.30 ± 1.12 . With a few inter-type individ-

uals, the distribution of these activity values was plotted in a tri-modal curve. These results suggest that the esterase D system in human red cells is qualitatively and even qualitatively controlled by genes.

A19. A Phylogeny for the Principal Alleles of Human Phosphoglucosmutase-1 (PGM_1):

Norio TAKAHASHI, Chiyoko SATOH, Naomi MASUNARI, Junko NISHIZAKI
and James V. NEEL (RERF, Hiroshima and Univ. Michigan)

It has been reported that PGM_1^1 and PGM_1^2 heretofore believed to be single alleles according to starch gel electrophoretic analysis, can each be classified into 2 different alleles by polyacrylamide thin layer gel isoelectric focusing (PAGIF). We reported PGM_1^7 is also classifiable into two alleles by PAGIF. This report describes the results obtained from the examination of the PGM_1^3 allele by PAGIF. Samples were obtained from a total of 28 individuals selected from participants in a study of "Genetic study of offspring of A-bomb survivors," who were known to have either PGM_1 1-3 or PGM_1 2-3 from a prior starch gel electrophoretic study. Of the 28 PGM_1^3 gene products subjected to PAGIF, 23 (82.1%) were PGM_1 3+ in type and 5 (17.9%) were PGM_1 3-. Thus, the 4 kinds of conventionally defined alleles were subdivided into 8 after PAGIF examination. On the basis of frequencies of these alleles, isoelectric points of allozymes produced by them and the geographical distribution of 4 "conventional" alleles, an allele phylogeny of PGM_1 can be constructed. This phylogeny postulates that 8 alleles may be explained by 3 alleles produced from 3 nucleotide substitutions plus 4 intragenic recombinations between these alleles. It should be possible to confirm this hypothesis by amino acid sequencing of the allozymes of PGM_1 and ultimately by DNA sequencing of the PGM_1 gene.

A20. Genetic Distance between Iran and East Asian Populations: Tsutomu MIYA-

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

Iranian had been influenced by both Europeans and Asians for a long time.

We surveyed several populations in East Asia in relatively large scale since the beginning of 1960s. In 1972, we observed Iranians (Mazandaranian and Guilanian) for several genetic polymorphic traits. Then we are trying to reveal the anthropogenetic position of Iranian by means of the genetic distances between East Asian populations and Europeans.

We used the following data to compute the genetic distance: Iran (Ohkura, Miyashita and Nakajima, 1972), Korea (Ohkura, Miyashita and Nakajima, 1973-1979), China (Ohkura and Nakajima *et al.*, 1967), Taiwan (Ohkura and Nakajima *et al.*, 1967), Japan (Nakajima, 1973; Shinoda *et al.*, 1970; Ishimoto, 1967), England (Mourant and Kopec, 1976).

The genetic distance were computed from the gene frequencies of ABO, MN, Rh (Cc, Ee), Hp and Catalase, by the method of Cavalli-Sforza and Edwards (1967). The distance from Japan to the other populations is shown as follows:

Japan—N. China—Korea—Taiwan—S. China—Iran—England
(0.047) (0.052) (0.087) (0.093) (0.155) (0.201)

Among these populations, the most distant population from Japan is England and the next is Iran. The order from other East Asian populations to Iran and England is almost same as Japan. So, Iran is located inbetween East Asia and England.

Increasing the number of kind of traits (ABO, MN, Ss, Rh-Cc, Ee, P, Duffy, Diego, Kidd, PGM, Hp, Gc and Catalase), the distances from Japan to Iran and from Iran to England are become more precisely as shown as follows:

Japan—Korea—Iran—England
(0.065) (0.270) (0.309)

Iran is more closer to England rather than East Asia.

A21. Distribution of Hp, Tf, Gc and Pi Polymorphisms in a Nepalese Population:

Isao YUASA (Dept. Legal Med., Tottori Univ. Sch. Med.), Yukio SANESHIGE (Dept. Med. Tech., Tottori Univ. Coll. Med. Care Tech.) and Naoyuki OKA-MOTO (Dept. Hyg. Tottori Univ. Sch. Med., Yonago)

Hp typing and Tf, Gc and Pi subtypings were performed of 144 serum samples from a Nepalese population (mainly Newars) in Dhapakhel, Katmanzu Valley, Nepal. *Hp*: The value of Hp^I allele frequency was 0.1771 in this sample, similar to the average value among Indians in India, who were characterized by low frequency of Hp^I allele. *Tf*: 5 of the 6 common subtypes determinable by Tf^{C1} , Tf^{C2} and Tf^{C3} alleles were observed, together with 3 samples having a more anodal band than Tf C1 band. This variant type (Tf Cnepal) was indistinguishable from Tf Pangwala type. Tf^P and Tf^{Cnepal} (if designated numerically, Tf^{C9}) may be race-specific in the Indian subcontinent just as some other Tf^C alleles are. *Gc*: The 6 common subtypes were observed in 143 serum samples. Another had double bands located between the Gc 1C2 and 1C4 bands in combination with the Gc 1S bands. *Pi*: All the samples belonged to the 6 common subtypes explainable by Pi^{M1} , Pi^{M2} and Pi^{M3} alleles. The obtained allele frequencies are as follows: $Hp^I=0.1771$, $Hp^2=0.8229$; $Tf^{C1}=0.7222$, $Tf^{C2}=0.2500$, $Tf^{C3}=0.0174$, $Tf^{Cnepal}/(Tf^{C9})=0.0104$; $Gc^{1F}=0.2448$, $Gc^{1S}=0.4825$, $Gc^2=0.2727$; $Pi^{M1}=0.6076$, $Pi^{M2}=0.2118$, $Pi^{M3}=0.1806$.

A22. First Japanese Example of the Rare Blood Phenotype Jk(a-b-) and Incidence of the Phenotype in Japanese Population: Masaaki MIZUI (Hiroshima Red Cross Blood Cent., Hiroshima), Eiichi UCHIYAMA (Okayama Red Cross Blood Cent., Okayama), Masaki KIKUCHI (Miyagi Red Cross Blood Cent., Sendai), Sunao MURATA (Saitama Red Cross Blood Cent., Saitama), Taiko SENO, Yasuto OKUBO, Hideo YAMAGUCHI (Osaka Red Cross Blood Cent., Osaka) and Isao YUASA (Dept. Legal Med., Tottori Univ. Sch. Med., Yonago)

The propositus is a 42-year-old male donor who visited the Hiroshima Red Cross Blood Center. His blood was brought to our notice when his serum contained an irregular antibody though he had not transfused. The antibody was identified as anti-Jk3, because his serum reacted well with all the cells tested except Jk(a-b-). His cells failed to react either with anti-Jk^a, anti-Jk^b or anti-Jk3 as expected.

The family study revealed that one of his sister was Jk(a-b-), but her serum contained no irregular antibody though she had been pregnant twice before. The parents of the propositus were first cousins. This is the first example of Jk(a-b-) found in Japanese.

Using the urea lysis test reported by Heaton and McLoughlin (1982), we screened random donors' cells for Jk(a-b-) phenotype and obtained following results: none in 25,000 Miyagi donors, none in 56,715 Saitama donors, one in 21,011 Osaka donors, one in 9,374 Okayama donors, three in 40,254 Hiroshima donors; five in 152,354 (0.003%) in all.

None of the newly detected Jk(a-b-) donors had anti-Jk3 in the serum. The parents of four of the five probands were consanguineous.

At present the genetic mechanism, an amorphic allele at the *Kidd* locus or a regulator gene which produces these Japanese examples of Jk(a-b-), is unknown.

A23. A Family Example of A_{int} which Demonstrate Different A Antigen Strength and Activity of A-Enzyme: Tamiko NAKAJIMA, Ryoko TAKAYANAGI, Shin YAZAWA, Tadahisa KOGURE, Ken FURUKAWA (Dept. Legal Med., Gunma Univ., Maebashi), Taiko SENO and Hideo YAMAGUCHI (Osaka Red Cross Blood Cent., Osaka)

Propositus (R.T.) and her mother (M.T.) were blood group A_{int}B and mother's sister (F.S.) was group A_{nit}. The agglutinability of the red cells of R.T. and M.T. against *Dolichos biflorus* (agglutinin titer 1 : 64) and *Falcata japonica* (agglutinin titer 1 : 64) lectins were 1 : 1 and 1 : 16, respectively. The agglutinability of F.S. red cells with the lectins was 1 : 4 and 1 : 16 respectively. Their red cells did not react with anti-Cad chicken serum. They belong to secretors and anti-A agglutination inhibition titer of saliva from R.T. was 1 : 5,120 while that of M.T. was 1 : 20. R.T. and M.T. secreted B and H substances in normal amount. F.S. secreted normally A and H substances in saliva. α -N-Acetylgalactosaminyltransferase activity of serum and red cell membranes were tested by

the transformation of group O red cells to A active cells and by the incorporation of radio-active *N*-acetylgalactosamine into H active oligosaccharides, lacto-*N*-fucopentose I and 2'-fucosyllactose in the presence of UDP-*N*-acetylgalactosamine (UDP-GalNAc). The A-enzyme activity of A_{int}B and A_{int} was between A₁ and A₂, and had optimum at pH 6.5-7.0. The enzyme activity was strongest in R.T. and there were no correlation between A antigen strength of red cells and saliva, and A-enzyme activity in serum and red cell membranes. Action of purified A-enzyme from group A₁ plasma on F.S. red cells in the presence of UDP-GalNAc resulted increase of agglutinability of the red cells with *Dolichos* lectin at the group A₁ level.

A24. 等電点電気泳動によって見いだされた尿中の糖タンパクの遺伝：岸絃一郎・滝沢久夫・山本 茂 (科警研・法医). Genetic Variants in Human Urinary Glycoproteins by Isoelectric Focusing: Koichiro KISHI, Hisao TAKIZAWA and Shigeru YAMAMOTO (Medico-Legal Sec., Natl. Res. Inst. Police Sci., Tokyo)

健康な東京在住者の 100 倍濃縮尿について pH 3 ないし 10 の範囲の等電点電気泳動を実施すると、pH 7 前後の領域に 3 本のバンド (A, B および C) が見いだされた。A バンドは、主バンドとこれより陰極側の副バンドとからなっていた。B バンドは A バンドよりも陽極側にあり、C バンドは A バンドより陰極側に位置していた。検査したすべての個体は A バンドを有していた。両親が A バンドのみをもつ 10 家族についてみると、子供 20 人はすべて A バンドのみを保持していた。Y 家では父、息子および娘が A および B バンドを有していたが、母は A バンドのみを有していた。N 家では父が A および C バンドを有していたが、母、息子および娘は A バンドのみを有していた。

以上の家族調査から、これらのバンドには遺伝性が認められた。また、これらのバンドはいずれもターマシーブルーおよび PAS 陽性であって、糖タンパクであろうと考えられた。そこで、これらのバンドを尿の糖タンパクにちなんで Ugl-A, -B および -C と名づけた。

A25. Vitamine-D binding protein (Gc) 亜型間にみられる血清濃度差：河合尚樹・松井清司・柴田和重 (大阪医大・法医). Concentration Difference of Vitamine-D Binding Protein (Gc) Subtypes among Normal and Liver Cirrhosis: Naoki KAWAI, Kiyoshi MATSUI, Kazushige SHIBATA (Dept. Legal Med., Osaka Med. Coll., Takatsuki)

われわれは、vitamine-D binding protein (Gc) の laser nephelometry による新しい定量法を開発し、780 名を亜型別に分類し、その定量値に関して t-検定を行った。その結果 1F, 1S, 1F-1S の 3 つの亜型間では差がなく 30~31 mg/dl を示したが、2 のホモ接合型では 26.6 mg/dl と有意な低値を示した ($p < 0.001$)。また、2-1F, 2-1S 型のヘテロ接合型では、28.7 と 28.6 mg/dl とその中間の値をとり両者に差は認めなかった。次に、肝硬変患者 170 名について、亜型別に検討した。亜型の分布は正常者とよく一致した。すべての亜型で低下を示したが、その減少の程度は亜型間で差を認め、1F, 1S, 1F-1S, 2 のそれぞれの亜型では約 5 mg/dl の減少を認めたのに対し、2-1F, 2-1S のヘテロ接合型では、その減少傾向は著しくなく、それぞれ 3.3 mg/dl と 2.0 mg/dl の低下しか示さなかった。

これは 1978 年 Brown らの報告と一致するが, Brown はみずからこの結果を少数例による誤差として意義を否定している。われわれは今回, 多数例を検討することにより, 肝硬変における亜型間の Gc 蛋白濃度の減少の程度に差があることを確認しえたものとする。

A26. α_1 -Antitrypsin Phenotypes in Japanese (IV) Description of Some New Pi Variants: Kazuhiko MIYAKE, Masao TORII, Kenzi ITOKAZU, Yoshimichi ITOH, Atsushi OHKUNI, Takashi NAKAMURA and Masami YAMANAKA (1st Dept. Med., Teikyo Univ. Sch. Med., Tokyo)

Pehnotypes of α_1 -antitrypsin have been determined by isoelectric focusing in serum samples from more than 2,000 healthy Japanese living in Tokyo. Gene frequencies for three subtypes of M, designated M1, M2, M3 were 0.714, 0.250 and 0.036 respectively, while rare variants were detected less than 0.5% in frequency and no Z gene was observed. A low incidence of non M alleles seems to be common in Asian countries as Fagerhol noted. There are more than 34 Pi alleles so far reported. We discovered some new Pi variants in Japanese. The nomenclature used for Pi was according to the International Pi Committee (*Hum. Genet.* 53, 429, 1980). 6 family pedigrees of propoiti with the following Pi phenotypes are reported: (1) Etok M1 (2) Etok M2 (3) Mbal P (4) Mbal Pfuk (5) M1 Pkyo (6) M1 Zhyo. Etok is located more anodal than E. Pfuk different from R migrates faster than Pkyo and P. Pkyo is close to P but appears to migrate a little faster than P. Pkyo is identical to S that obtained with starch gel electrophoresis. Zhyo is demonstrated far more cathodal than Z and has not been confirmed if it is different from the known variant, Zpra. Mbal is very close to M2 by isoelectric focusing and different to M2 by starch gel electrophoresis. Etok, Mbal, Pfuk and Pkyo samples were submitted to the reference laboratories. All these variants are not deficient types.

A27. Studies on Complement Deficiencies in Blood Donors in Osaka Area of Japan: Keiji YOSHIMURA, Yasuo FUKUMORI, Shiro OHNOKI, Yasuto OKUBO, Hideo YAMAGUCHI, Masayoshi TANAKA (Osaka Red Cross Blood Cent., Osaka), Yohji AKAGAKI and Shinya INAI (Dept. Clin. Pathol., Osaka Med. Coll., Takatsuki)

The incidence of the complement deficiency in 52,175 blood donors was investigated by the microtitration method of hemolytic complement using sucrose gelatin veronal buffer (SGVB). No hemolysis was observed in 283 serum specimens, and 55 of them were identified as C9 deficiency by hemolytic assay of whole complement (CH50) and C9, and immunochemical analysis of C9. CH50 units of these sera were 12.1 ± 3.8 , which is 25% of the normal value of CH50. C9 in these sera was not detected by immunochemical analysis and hemolytic assay. All the donors with C9 deficiency were in good health. The

family studies on C9 deficiency revealed that the mode of inheritance of the C9 deficiency is considered to be autosomal co-dominant.

Moreover, five of the 52,175 donors were suspected as C7 deficiency and two C8 deficiency. CH50 units of these sera were lower than 2.0. In each of the complement deficient sera, C7 or C8 was detected by neither hemolytic assay nor immunochemical analysis. The C7 or C8 deficient donors were in good health. Further studies are being carried out about C7 or C8 deficient donors.

The results obtained indicated that the incidence of C9 deficiency in Osaka donors might be 0.105%.

A28. Genetic Polymorphism of the Sixth Component of Human Complement (C6) in Japanese: Katsushi TOKUNAGA, Keiichi OMOTO (Dept. Anthropol., Univ. Tokyo), Yasushi YUKIYAMA (Dept. Med. Physic. Therapy., Univ. Tokyo, Tokyo) and Takeo JUJI (Blood Transfus. Service, Tokyo Women's Med. Coll., Tokyo)

Polymorphism of the sixth component of human complement (C6) was investigated in Japanese. After the separation by thin layer slab polyacrylamide gel isoelectric focusing (pH 5-8), agarose gel in GVB²⁺ containing C6 deficient rabbit serum and EAC 14 cells was overlaid for specific hemolytic detection. The results of typing of a family material consisting of 17 matings with 56 offsprings supported that C6 polymorphism is controlled by autosomal codominant alleles. A total of 188 unrelated healthy adult Japanese living in Tokyo were typed for C6. Five common and three rare phenotypes were observed, and these were considered to be controlled by three common and two rare alleles. Judging from hemolytic patterns, these variants were tentatively called C6 A, C6 B, C6 B2J, C6 A3J, and C6 MJ, respectively. The allele frequencies were estimated as follows: C6*A=0.439, C6*B=0.463, C6*B2J=0.093, C6*A3J=0.003, C6*MJ=0.003. The observed numbers of phenotypes were in agreement with those expected under Hardy-Weinberg's law ($\chi^2=1.28$, 2 d.f., $0.6 > p > 0.5$). At least three common alleles (C6*A, C6*B, and C6*B2J) occur in Japanese, while two (C6*A and C6*B) in Caucasoid populations.

A29. Cryptomeria Pollinosis: A Model of the Genetic Control of Immune Response in Man: Masahiko MUTO, Kikuo TSUKAMOTO, Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ.) and Yoza SAITO (Dept. Otolaryn., Tokyo Med. Dent. Univ., Tokyo)

Analysis of mode of inheritance for the susceptibility to cryptomeria pollinosis (CP) by affected sib-pair method and Morton's maximum likelihood scoring method revealed that CP was controlled by a single recessive gene and the disease susceptibility gene might

be linked to HLA. The linkage study with the sequential analysis described by Morton revealed that close linkage between HLA and CP (maximum lod score = 3.045 at $\theta = 0.11$). All these results show that an HLA-linked dominant gene controls the resistance to CP or nonresponse to cryptomeria pollen antigen. Also the patients with CP showed a statistical association with HLA-Bw44-8wDRw6y-MB3 (primary association was with MB3; relative risk = 3.30, $p < 0.03$).

Furthermore *in vitro* immune response (IgE and IgG) detected by radioimmunoassay, it was demonstrated that the nonresponders have active suppressor T cells (Leu-2a⁺) which completely suppress IgE and IgG response of HLA haploidentical responder lymphocytes to cryptomeria pollen antigen. Therefore the HLA-linked gene controlling the nonresponse to cryptomeria pollen antigen and the resistance to CP is *Is*-gene.

A30. Analysis of the HLA-D Region Antigen from Dw2 or Dw12 Homozygous B Lymphoblastoid Cell Lines: Toshio SONE (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo), Toshinao TAKENOUCHI, Akemi WAKISAKA, Miki AIZAWA (1st Dept. Pathol., Hokkaido Univ., Hokkaido) and Takehiko SASAZUKI (Dept. Hum. Genet., Tokyo Med. Dent. Univ., Tokyo)

HLA-D region antigen from HLA-Dw2 or Dw12 homozygous B lymphoblastoid cell lines were compared by two dimensional gel electrophoresis. Dw2 and Dw12 are distinct in MLC, but both B cells typed as Dw2 or Dw12 are typed as HLA-DR2 in serology. Monoclonal antibody HU-4 immunoprecipitated the DR antigen and that inhibited for stimulation in MLC between Dw2 and Dw12.

HLA-D region antigen immunoprecipitated with HU-4 from Dw2 or Dw12 homozygous B lymphoblastoid cell lines were composed of an α chain and two distinct β chains ($\beta 1$ and $\beta 2$ chain) from the results of 8 hr labeling and 30 min pulse labeling. α chain and $\beta 1$ chain from Dw2 were identical with those from Dw12 respectively but $\beta 2$ chain from both cell lines were distinct in polypeptide level and not on the step of N-linked glycosylation from the analysis of mixture of D region antigen from tunicamycin treated cell lines. $\beta 2$ chain can stimulate MLC reaction between Dw2 and Dw12.

These results reveals that HLA-D region is constructed with at least two distinct loci in which structural genes of $\beta 1$ and $\beta 2$ chains are coded.

A31. Differential Recognition of the Serologically Defined HLA-A2 Antigen by Allo-genetic Cytotoxic T Cells: Satoshi HORAI (Dept. Anthropol., Univ. Tokyo, Tokyo), Jan J. van der POEL, Els GOULMY and J.J. van ROOD (Dept. Immunohaematol., Univ. Hosp., Leiden, the Netherlands)

Human alloimmune cytotoxic T cells, sensitized selectively against the HLA-A2 antigen, were tested on a panel of selected target cells (58 HLA-A2 positive and 28 HLA-A2 nega-

tive). A clear cut bimodal distribution of positive and negative target was observed, and five HLA-A2 positive outlier cells could be identified. These outlier cells were only weakly lysed by HLA-A2 specific CTLs, although they were serologically indistinguishable from the other HLA-A2 positive, strongly lysed target cells. Furthermore, it was found that the outlier cells were poor could target inhibitors in contrast to the other HLA-A2 positive target cells, which showed adequate inhibition of specific lysis of HLA-A2 positive target cells. Absorption studies of HLA-A2 antisera by outlier and control cells showed that all HLA-A2 positive cells absorbed the anti-HLA-A2 activity. Population studies indicate that the frequency of such HLA-A2 outlier cells may be approximately 10%. Furthermore, the outlier cells can be divided into different subsets.

A32. Analysis of the Immune Suppression Gene to the Streptococcal Cell Wall Antigen (Is-SCW) in Man: Yasuharu NISHIMURA and Takehiko SASAZUKI (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

We have already reported the genetic control of the immune response to the streptococcal cell wall (SCW) antigen in man and elucidated the following points. 1) The distribution of the immune response of healthy adults to the SCW antigen could be divided into high and low responder groups. 2) Low responsiveness was controlled by an HLA-linked dominant gene (Is-SCW). 3) Low responsiveness was determined on T cell level.

We further analyzed the low responsiveness expressed on T cells. Unfractionated T cells from a low responder completely suppressed the immune response of high responder T cells to the SCW antigen. This immune suppression was mediated by Leu-2a positive suppressor T cells. Low responder T cells showed marked immune response to the SCW antigen after the elimination of Leu-2a positive suppressor T cells. Low responsiveness to the SCW antigen was, therefore, controlled by an HLA-linked dominant gene through Leu-2a positive suppressor T cells.

Some high responders had Leu-2a positive suppressor T cells which could partially suppress the immune response of autologous and allogeneic high responder Leu-2a negative T cells to the SCW antigen, but these Leu-2a negative T cells still showed phenotypically high immune response at the existence of high responder suppressor T cells. Suppressor T cells from high and low responders are, therefore, different and the Is-SCW controlled the induction of low responder type suppressor T cells.

Is-SCW was in linkage disequilibrium with some alleles of HLA-D region but not with any alleles of HLA-A,B,C or SB indicating that the Is-SCW should be mapped in HLA-D region.

A33. Analysis of Human Ia Antigen Using Two Dimensional Gel Electrophoresis.

I. MT Antigen System: Manabu SUZUKI, Ryozauro MUKAI, Toshio YABE, Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki), Takeo JUJI (Dept. Blood Transfus., Tokyo Women's Med. Coll., Tokyo) and Hiroo MAEDA (Dept. Blood Transfus., Tokyo Univ. Hosp., Tokyo)

HLA-D/DR region corresponds to H-2 I region of mouse that has been divided into four subregions and only DR antigen system has been approved.

Recently it is suggested that MT and MB antigen systems defined serologically as DR supertypic specificity is present. MT3 antigen is strongly associated with DR4, DR7, DRw9 and DRw10. However, it is not elucidated yet whether MT3 antigenic site is present on the DR molecule as a supertypic specificity or on a molecule distinct from DR molecule. In order to clarify whether MT3 antigen is distinct from DR molecule or not, following experiments were performed.

Three human B lymphoid cell lines, Wa (DR4, MT3) Pit (DR7, MT3) Mae (DRw9, MT3), were used to prepare MT3 antigen. The three cell lines were labeled with [³⁵S]-methionine metabolically and solubilized in NET buffer containing 0.5% NP-40. Plasma membranes were also prepared from Wa cells by sucrose density gradient centrifugation and solubilized in NET buffer containing 1% NP-40. Glycoproteins of the plasma membranes were purified by Lentil-lectin affinity chromatography and labeled with ¹²⁵I by Chloramin T method. The antigen preparations reacted with both anti human Ia monoclonal antibodies and alloantisera against DR4, DR7, DRw9 and MT3. The antigen-antibody complex was absorbed with Protein A Sepharose and solubilized with 9.5 M urea, 5% 2-ME, 2% NP-40. The solubilized antigen was analysed using two dimensional gel electrophoresis and autoradiography.

Analysis of two dimensional gel electrophoresis indicated that the positions of DR light chains (Mr, 33-30k) are different from each other among DR4, DR7 and DRw9.

Among them, pI of the light chains of DRw9 was most basic, that of DR7 was most acidic, and that of DR4 was intermediate. On the contrary, DR heavy chain (Mr, 33-34k) cannot be distinguished among them. MT3 molecule could be identified as one heavy chain spot (Mr, 33-34k) and a set of several light chain spots (Mr, 29-30k) in each of three cell lines. The pI of MT3 light chain was more acidic than that of three DR light chains. The data indicate that MT3 antigen is a new Ia molecule distinct from DR antigen.

A34. Analysis of Human Ia Antigenic System by Two-Dimensional Gel Electrophoresis. II. Identification of TB21 Antigen: Ryoaburo MUKAI (Dept. Hum. Genet., Univ. Tsukuba), Hiroo MAEDA (Dept. Blood Transfus., Univ. Tokyo, Tokyo), Toshio YABE, Manabu SUZUKI, Hideo HAMAGUCHI (Dept. Hum. Genet., Univ. Tsukuba, Ibaraki) and Takeo JUJI (Dept. Blood Transfus., Tokyo Women's Med. Coll., Tokyo)

In the accompanying paper we reported that the light chain of MT3 antigen is distinct from that of DR antigen, using DR4, DR7 and DRw9 homozygous 3 cell lines. The light chain of MT3 from these 3 cell lines had almost same isoelectric points and molecular weight.

Recently, several groups have serologically defined multiple Ia-like antigens. TB21 antigen is one of such antigens which is different from MB and MT antigens. It has a strong association with HLA-B40 and DRw9 but a weak association with DR 4, 5 and 7.

To identify TB21 antigen, we used 0.5% NP-40 lysate of B-lymphoid cell line which were metabolically labeled with [³⁵S]methionine for 4 hr. Cellular extracts from different B-lymphoid cell lines were reacted with monoclonal antibody or alloantiserum at 5°C for 2–12 hr depending on the titer of antibody. Immune complexes were purified and radioactive antigens were analyzed by O'Farrells NEPHGE 2-dimensional electrophoresis followed by fluorography. Radioactively labeled cell extracts were prepared from 5 B-lymphoid cell lines: Mae (DRw9, DRw9), KCCL (DR5, DRw9), OV-1 (DR1, DRNJ-2), Sh (DRNJ-2, DRNJ-2), Wa (DR4, DR4). All the DR antigens in these cell lines were identified by the use of DR-framework monoclonal antibodies and/or alloantiserum. MT3 antigen in Mae cell line and KCCL cell line were identified by alloantiserum. These 5 different cell extracts were also reacted with 2 different lots of alloanti TB21 serum. These alloanti TB21 serum precipitated essentially same Ia-like heavy and light chain (apparent m.w. 33,000 and 28,000, respectively together with actin, P35 polypeptide and Ii). Three to four TB21 light chain spots which were distinct from those of corresponding DR and MT3 antigen were observed. Spots of TB21 light chain obtained from Mae cell line differed from those obtained with KCCL, OV-1 and Sh cell lines as to position in pH gradient and molecular weight. Spots of TB21 light chain obtained with KCCL, OV-1 and Sh cell lines overlapped each other. Wa cell line which is negative for TB21 antigen did not have any corresponding spots specific for anti TB21 serum. Besides the TB21 light chain spots common with OV-1 and Sh cell line, KCCL cell line had 4 additional TB21 light chain spots. These extra 4 spots in KCCL cell line seems to be identical to TB21 light chain spots obtained with Mae cell line.

These results indicate that TB21 light chains were distinct from MT3, DR5 and DRw9 light chains. These results also suggest that there are heterogeneity in light chain of TB21.

- A35. 免疫グロブリンアロタイプによる IgG サブクラスの決定: 宮崎時子・松本秀雄・豊増 翼 (大阪医大・法医), 桜林郁之介 (自治医大・臨床病理). *Determination of IgG Subclasses Using Human Immunoglobulin Allotypes: T. MIYAZAKI, H. MATSUMOTO, T. TOYOMASU (Dept. Legal Med., Osaka Med. Sch., Takatsuki) and I. SAKURABAYASHI (Dept. Clin. Pathol., Jichi Med. Sch., Tochigi)***

異なる IgG サブクラスが, それぞれ違った生物学的な性質をもち, 特異的な抗原には特定のサブクラスに属する, すなわち限られたアロタイプをもつ抗体が対応することが知られている. これは抗体特異性を左右する IgG・V 部遺伝子が特異的な IgG サブクラスと関連することによると考えられ, ある一つのサブクラスの中で特異的なアロタイプ決定基と関連することが十分に考えられる.

われわれは, IgG サブクラスとそれがもつ遺伝マーカーと疾病との相関を明らかにする目的で, まず骨髄腫蛋白質における IgG サブクラスと Gm, Km アロタイプの分布頻度を検索した. 特定のサブクラスに特定のアロタイプが局在することを利用して, IgG サブクラスを決定したが, Gm 型の検査は通常の血球凝集阻止試験によって, $\gamma 2$ と $\gamma 4$ の決定には CrCl_3 法と, $\gamma 2$, $\gamma 4$ 感作溶血性貧血患者血球を用いた. 312 例の骨髄腫蛋白質について $\gamma 1$ (250 例); az (186), af (26), axz (38); $\gamma 2$ (35 例); n+(1), n-(34); $\gamma 3$ (9 例); g (8), $b^{035}\text{st}$ (1); $\gamma 4$ (18), κ 型 (66) λ 型 (48), の成績を得た. $\gamma 1$ では az が圧倒的に高く, af, axz は比較的少ない. 欧米人と対照的に必ず a と f は一緒にみられる. L 鎖によつては κ , λ 比はほぼ 1:1 といえる.

重症複合型免疫不全症の骨髄移植の 2 例について, その成果判定にこの方法を用いたが, 移植骨髄の生着と患者自身の細胞の再構築いかんの判定にきわめて有効であることを確かめえた.

- A36. A Neutrophil Adhesion Abnormality with an Autosomal Recessive Inheritance: Kunihiko KOBAYASHI, Kyoko FUJITA and Fumiko OKINO (Dept. Pediat., Yamaguchi Univ., Yamaguchi)**

A four-month-old Japanese girl with persistent navel infection by *Pseudomonas aeruginosa* had a high peripheral blood white cell count of $120 \times 10^3/\text{mm}^3$ with mature neutrophils. Routine neutrophil function tests revealed a lack of chemotaxis and a marked impairment of adhesion (4.6% in the patient vs. 65.4% in age-matched controls). The adhesion test in the mother showed a slight reduction of 76.2%, while that in the father was 80.4% (94% in adult controls). Analyses of the neutrophil membrane proteins disclosed that a protein with a molecular weight of 110×10^3 daltons was missing in the patient's neutrophils, while it was present but reduced in both parents' neutrophils. These results suggest that the disease was inherited in an autosomal recessive fashion.

The neutrophil adhesion abnormality, resulting from the lack of the neutrophil membrane protein, has been reported only once, in a 5-year-old American boy (Crowley *et al.*, 1980). The disease, however, was inherited as an X-linked. Thus, the disease in our patient is similar to, but different from, that reported by Crowley *et al.*

A37. Immunogenetic Analysis of Leprosy II: Ikuo KIKUCHI, Takehiko SASAZUKI
(Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), Toshiharu OZAWA (Natl. Inst. Leprosy Res., Higashimurayama), Kiyotaka SANADA and Masanori KOSEKI (Natl. Sanat. Tama-Zenshoen., Higashimurayama)

Genetic control of the clinical course of infection with *Mycobacterium leprae* was investigated using 43 unrelated patients with leprosy (23 tuberculoid patients and 20 lepromatous patients) and 54 members of 14 multiple case families.

In 23 unrelated patients with tuberculoid leprosy, HLA-DR2 was significantly increased (relative risk=3.08 $\chi^2=5.09$) compared to normal controls, and HLA-MT1 was also significantly increased (relative risk=9.30, $\chi^2=6.18$).

In 20 unrelated patients with lepromatous leprosy, neither HLA-DR2 nor MT1 was significantly increased (relative risk=2.13, $\chi^2=1.64$, relative risk=1.48, $\chi^2=0.18$, respectively).

Multiple case families with leprosy were analyzed by affected sibpairs method. Affected sibpairs who apparently shared at least one HLA haplotype but who could not be unequivocally assigned as sharing two, one or zero HLA haplotype identical by descent were divided with weight according to the proportion between the definite case sharing two, one or zero. The "adjusted total" was used in the analysis according to the affected sibpairs method. Out of 13 affected sibpairs with lepromatous leprosy 7 shared two HLA haplotypes and 6 shared one HLA haplotype which differed from the random distribution ($\chi^2=7.60$, DF=2, $p<0.025$). This method could not distinguish between the recessive model (gf=0.45) and dominant model (gf=0.14) for the HLA-linked disease susceptibility locus in lepromatous leprosy.

These observation strongly suggested that an HLA-linked gene controlled the clinical course of infection with *Mycobacterium leprae*.

A38. The Survey of Abnormal Hemoglobin in Kobe District: Shunichi SHIMASAKI, Iwao IUCHI, Kazuo HIDAKA (Dept. Biochem., Kawasaki Med. Sch., Kurashiki) and Wataru MIZUTA (Kobe Municipal Cent. Hosp., Kobe)

Seven hemoglobin variants from a total of 23,482 specimens were detected by the hemoglobinopathy survey in Kobe district during the period from May, 1981 to October, 1982. Three variants were identified as Hb Ube 2 ($\alpha 68$ Asn $\rightarrow$ Asp), Hb Syracuse ($\beta 143$ His $\rightarrow$ Pro) and a new Hb Suma ($\alpha 11$ Lys $\rightarrow$ Asn) which has not been reported previously. The others were the two variants of β chain anomalies and the two variants of α chain anomalies. Their structural characterizations are in progress.

Since first discovery of Hb Ube 2 in the Japanese in 1960, several cases have been detected from different districts. Therefore, this variant seems to be sporadically distributed

among the Japanese. The carrier of Hb Ube 2 in Kobe revealed neither clinical symptoms nor hematological abnormalities and the normal functional characteristics as reported in other cases. Hb Syracuse is a first example detected in the Japanese. It is characterized by a high oxygen affinity and erythrocythemia to the carrier. The functional alterations are elucidated from the molecular bases of hemoglobin because the position of $\beta 143$ is one of 2,3-DPG binding sites and contributed much to $R \rightleftharpoons T$ allosteric change. Hb Suma gave negative isopropanol precipitation test. Its functional study demonstrated slightly increased oxygen affinity, slightly decreased alkaline Bohr effect and normal 2,3-DPG effects.

The frequency of abnormal hemoglobin detected in Kobe district was $\frac{1}{3,400}$ indicating similar values to those of other districts in Japan $\left(\frac{1}{2,700} - \frac{1}{4,000} \right)$.

A39. Hemoglobin Munakata: $\alpha 90$ (FG2) Lysine $\rightarrow$ Methionine—A New Variant Found in a Japanese Family: Jun SUGIHARA, Takashi IMAMURA, Toshikazu MATSUO, Masaaki KAGIMOTO, Ikuo SUMIDA, Yoko OKAZAKI and Toshiyuki YANASE (1st Dept. Med., Kyushu Univ., Fukuoka)

In a survey of blood specimens obtained from patients attending clinics in Kyushu University Hospital, a new variant of Hb A was found. The propositus was an apparently healthy Japanese man aged 55, living in Munakata, Fukuoka Prefecture. Hematological findings were normal. Thin-layer starch gel electrophoresis of hemolysates revealed a variant moving faster than Hb A. This variant comprised 18.2% of the total hemoglobins, and was shown to be stable on isopropanol stability test. Hemoglobins A₂ and Hb F were 2.7% and 0% of the total, respectively. Abnormal component was isolated by DE-52 column chromatography. Tryptic peptides of the aminoethylated α -chains of the variant were separated on HPLC with reversed phase ODS Sil C₁₈ column. The amino acid analysis of the peptide corresponding to $\alpha T9$ revealed that a methionine residue substituted for a lysine residue at position 90 of the α chain. As a result of this substitution, this peptide also contained two additional residues, leucine and arginine, from $\alpha T10$ peptide. This variant was designated as Hb Munakata. Slightly increased oxygen affinity was associated with this amino acid substitution. Both the Bohr effect and the heme-heme interaction were normal. The difference is log P1/2 was 0.07 mmHg, in 0.05 M bistris-0.1 M NaCl-1 mM EDTA buffer, pH 7.29.

- A40. Genetic Polymorphisms in the Nucleotide Sequence of the β -Like Globin Genes for Japanese Individuals and Patients with β - or δ -Thalassemia:** Akinori KIMURA, Takashi IMAMURA, Eiji MATSUNAGA, Yoshiro OHTA, Shigeru FUJITA (1st Dept. Med., Ehime Univ., Ehime), Toshikazu MATSUO, Jun SUGIHARA, Takenori NAKAMURA, Toshiyuki YANASE and Yasunori TAKAGI* (1st Dept. Med. and *1st Dept. Biochem., Kyushu Univ., Fukuoka)

We present the results of a detailed comparison of the restriction endonuclease cleavage maps for the β -like globin genes for 14 healthy Japanese individuals, 2 patients with heterozygous β -thalassemia, and 3 with homozygous δ -thalassemia. The globin genes are isolated from the gene libraries of a normal subject and a δ -thalassemia homozygote, and the complete nucleotide sequences of the region encoding δ -globin gene and 5'- and 3'-flanking sequences are compared. The linkage arrangements of the five embryonic and adult β -like globin genes and two other pseudogenes either for normal or for thalassemia subjects are consistent with its identifications for other ethnics described so far: neither deletion nor insertion of nucleotides can be detected by hybridization to the cloned cDNA probes. A Hind III site in the γ -gene region showed sequence polymorphism. Comparison of the nucleotide sequence of δ -globin gene with that of cloned pH δ 1 gene (Maniatis *et al.*, 1980) showed single base substitutions in the large intervening sequence at positions 137, 151, 186, 188, 291, 292 and 540, single base insertions at positions 339 and 823, single deletion at 548, and a duplication of 9 bp between positions 651 and 659. Also, the 3'-flanking sequence has two single base substitutions at positions 51 and 98 3' to the AATAAA sequence, respectively. All these changes, however, are also detected in the δ -globin gene for normal Japanese control subject. Therefore, they might represent genetic polymorphisms in Japanese population, and the aberrant expression of δ -globin gene in homozygous δ -thalassemia might be due to a mutation outside the δ -gene region.

- A41. The First Family of Fletcher Factor Abnormality in Japan. Two Homozygotes and Twelve Heterozygotes:** Toshio SHIGEKIYO, Shigenori KAWAUCHI, Yujiro HIRAI, Osamu FUJINO, Akira SHIRAKAMI (1st Dept. Intern. Med., Univ. Tokushima) and Kazuo MIYOSHI (1st Dept. Intern. Med., Univ. Tokushima, Tokushima, and Okinaka Memorial Inst. Med. Res., Tokyo)

We reported a family (Nishioka family) of Fletcher factor abnormality found in Tokushima district. The proband (K.N., 47-year-old male) was subjected to investigations because of mild nasal bleeding and prolonged coagulation time found at check-up before the operation for chronic sinusitis. No hemorrhagic diathesis was found in other family members. The proband and his younger sister (C.M., 42 y.) were homozygotes born of consanguineous marriage of cousins. Twelve heterozygotes including their parents and

all four children (two children each) of two homozygotes were found over three generations. Inheritance was autosomal recessive.

Fletcher factor activity was 0.2% and 1% in the two homozygotes and ranged 23–50% among twelve heterozygotes. Fletcher factor antigen was 25% and 21% in the homozygotes, respectively, and ranged 50–76% among heterozygotes. Thus, Fletcher factor in this family was 'abnormal' Fletcher factor with relative activity (activity/antigen) of 0.01 to 0.05.

Since the first report of Hathaway in 1965, Fletcher factor deficiency was reported in 16 cases of 11 families, including 12 cases of 9 families in America, 1 case of 1 family in Nigeria and 3 cases of 1 family in Spain. Five of these cases could be called Fletcher factor abnormality with some antigenicity. This Nishioka family with two homozygotes and twelve heterozygotes was the first family of Fletcher factor deficiency (abnormality) in Japan and among the yellow race. This is also the first family with extensive investigations of Fletcher factor abnormality among family members.

A42. Red Cell Adenylate Kinase Deficiency Associated with Hereditary Nonspherocytic Hemolytic Anemia: Hisaichi FUJII, Kenzaburo TANI and Shiro MIWA
(Dept. Intern. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

We found a case of red cell adenylate kinase (AK) deficiency associated with hereditary hemolytic anemia. The proband is a 10-year-old Japanese girl. Her physical and mental development was normal. She showed moderate to mild hemolytic anemia since the neonatal period, and hepatosplenomegaly. The red cell AK activity was 44% of normal. Contents of red cell glycolytic intermediates and adenine nucleotides were normal when compared with a comparable reticulocyte-rich control. Glucose consumption and lactate formation were normal. Hexose monophosphate shunt activity was somewhat lower than that of a comparable reticulocyte-rich control. There was no significant differences in the contents of adenine nucleotides between the younger and older red cells of the patient. Enzymatic characterization using hemolysate revealed that the patient's AK had an increased Michaelis constant for ADP and slight thermal-instability. The patient's enzyme migrated approximately half-way between the AK 1 and AK 2 position on starch-gel electrophoresis. The parents were second cousins and the maternal grandparents were first cousins. Her mother, younger sister and maternal grandfather showed decreased red cell AK activity not associated with any abnormality in electrophoretic and kinetic properties. They were hematologically normal. Mode of inheritance of this disorder, at least in this case, is obscure. The mechanism of hemolysis observed in the proband might be due to a structural gene mutation which caused altered electrophoretic and kinetic properties.

A43. Two Kindreds of Phosphofructokinase Deficiency Associated with Congenital Nonspherocytic Hemolytic Anemia Found in Japan: Kenzaburo TANI, Hisaichi FUJII and Shiro MIWA (Dept. Intern. Med., Inst. Med. Sci., Univ. Tokyo, Tokyo)

Two kindreds of phosphofructokinase (PFK) deficiency associated with congenital non-spherocytic hemolytic anemia and mild myopathy was found in Japan.

Case 1 is a 35-year-old Japanese male with hemolytic anemia. As exercise induced abdominal pain and fever, he had not even try to do vigorous exercise since his childhood. Laboratory data showed moderately compensated hemolytic anemia. Family studies showed that parents were first cousins.

Case 2 is a 19-year-old Japanese male with hemolytic anemia and mild myopathy. Blood examinations showed well-compensated hemolytic anemia. No consanguinity was apparent in his family. Case 3 is one of his sisters. She also had hemolytic anemia and mild myopathy.

In erythroenzymatic studies, red cell PFK activities were decreased to about a half of normal in these cases. Forearm ischemic exercise tests showed no increase of lactate after the exercise in both probands' venous blood. We analyzed the partial purified normal red cell PFK, liver PFK and muscle PFK. Km ATP, F6P and pH optimum showed no remarkable differences in these two probands compared with normal controls. However, thermal stability tests showed moderately decreased activity in Case 2. Chromatographic separation and starch gel electrophoresis showed no demonstrable muscle-type PFK in both probands' red cell PFK. Muscle biopsies were performed on both probands. Muscle PFK activity was decreased to about a half of normal in Case 1. This enzyme was very thermo-labile and had normal antigenicity. No accumulations of glycolytic intermediates were not demonstrated in the muscle. Muscle PFK activity was decreased to a few percents of normal in Case 2. Exact analyses will be done in other institute. In summary, unstable muscle-type PFK is considered to be a pathogenic cause in Case 1, whereas defect of muscle-type PFK might be a pathogenic cause in Case 2.

A44. 遺伝性膵外分泌不全症：長野省吾・越和子・永井文作・川口真男・提島俊一・富田雅枝・荒島真一郎（北大・小児）. Hereditary Exocrine Pancreatic Insufficiency: S. NAGANO, K. KOSHI, B. NAGAI, M. KAWAGUCHI, S. SAGESHIMA, M. TOMITA and S. ARASHIMA (Dept. Pediat., Hokkaido Univ., Sapporo)

膵線維嚢胞症は、汗電解質異常、膵外分泌機能不全、慢性呼吸器症状をトリアスとする常染色体性劣性の遺伝性疾患である。わが国においては、1951 年以来、膵嚢胞線維症、胎便性腸閉塞等の病名で報告されているが、その数は 50 例に満たない。また、これらの報告のうち、汗電解質、膵外分泌機能に関するデータの完全なものは少ない。

また、シュバックマン症候群は、膵外分泌不全、骨髓機能障害（主として好中球減少）、ときとし

て、骨幹端性異骨症を伴う、やはり常染色体劣性の遺伝性腓外分泌機能不全症である。本疾患のわが国での報告は数例にすぎない。

今回、高温環境下で多量の発汗をしたことから著しい低電解質血症をきたし、食欲不振、体重減少を示した 9 カ月男児。離乳開始期より脂肪性下痢、体重増加不良が出現、1 歳半頃より呼吸器症状が目立つようになった 1 歳 1 カ月女児、2 例の腓線維嚢胞症。

および、腹部膨満、低身長、好中球減少症を示した 1 歳 1 カ月女児のシュバックマン症候群を経験した。以上の症例につき、文献的考察を加え、臨床経過、腓外分泌機能検査結果、脂肪吸収試験、PFD テスト、パンクレオザイミン-セクレチンテスト、汗電解質 (ピロカルピンイオントフォレーシス) につき報告する。

A45. Two Cases of Lysinuric Protein Intolerance: Takeo YOSHIMURA, Motohiro KATO, Ikuo GOTO and Yoshigoro KUROIWA (Dept. Neurol., Kyushu Univ., Fukuoka)

Two cases of lysinuric protein intolerance were reported. The patients, 14-year-old male (Case 1) and 12-year-old female (Case 2), were siblings in a family. Both cases had growth retardation and aversion to protein-rich food. Case 1 has had recurrent attacks of loss of consciousness since age of 5.

The urinary excretions of lysine, arginine and ornithine were markedly increased and their plasma concentrations were decreased. L-alanine loading test showed increased blood ammonia. The corresponding rise in blood urea nitrogen was abnormally low or absent. Addition of L-arginine to L-alanine loading prevented hyperammonemia and normalized the rise in blood urea nitrogen. These data suggested that aversion to protein and loss of consciousness were due to metabolic disturbance, chiefly hyperammonemia.

Growth retardation was thought to be a result of protein deficiency or pituitary insufficiency. In Case 1, secretion of growth hormone (GH) was abnormally low in insulin loading and the lower limit of normal range in glucagon-propranolol loading. However, arginine loading showed normal response. During treatment with arginine, GH was good response in insulin or glucagon-propranolol loading. There is a possibility that their dwarfism is due to hyposecretion of GH caused by deficiency of arginine.

Their parents were normal in physical and biochemical examinations.

Our cases is autosomal recessive trait without amino aciduria in heterozygotes.

A46. One-Month-Old Girl with β -Glucuronidase Deficiency (Mucopolysaccharidosis Type VII): Toshiaki TAGA, Takashi KUWABARA, Kuang-Chang HUANG, Hiroshi ICHIHASHI, Kazuko SUKEGAWA, Tadao ORII (Dept. Pediat., Gifu Univ., Gifu) and Ikuo IZEKI (Nagahama Red Cross Hosp., Nagahama)

Mucopolysaccharidosis type VII (β -glucuronidase deficiency) was first clinically observed by Sly *et al.* (1973) in a young negro child, and although only 12 cases have been reported

to date, considerable phenotypic variation, as well as the excretion pattern of urinary mucopolysaccharides, has already been observed, much in other lysosomal diseases.

We reported the clinical, cytological and biochemical findings in a one-month-old girl, in whom β -glucuronidase deficiency was diagnosed after the granulations of blood leukocytes were discovered during a routine hematological examination at the 4 days of age.

She presented with relatively mild symptoms including hepatosplenomegaly. Facial features were not coarse and no neurological abnormalities were present.

Urinary analysis revealed an increased excretion of mucopolysaccharides, especially chondroitin 4- and 6-sulfates.

In cultured skin fibroblasts, lymphocytes and serum, β -glucuronidase activity toward 4-methyl-umbelliferyl substrate was virtually absent.

Both parents showed an intermediate level of the enzyme activity, which suggested heterozygosity.

Although the disorder is caused by inactivity of single enzyme, the clinical expression and the excretion pattern of urinary mucopolysaccharides vary from case to case.

The results were compared with the 12 cases described in the literature.

A47. Clinical and Biochemical Heterogeneities of Hurler-Scheie Compound: T. TOKORO and Y. ETO (Dept. Pediat., Tokyo Jikei Univ. Sch. Med., Tokyo)

Recently, the mucopolysaccharidosis has been classified into several groups according to their defect of enzymes. Even in alpha-iduronidase deficiency, clinical variations have been noticed; Hurler syndrome, Hurler-Scheie syndrome and Scheie syndrome. However, their exact nature of biochemical differences among these disorders has not been identified. We experienced two cases with Hurler-Scheie syndrome. Radiological and clinical features in these cases were almost intermediate between Hurler and Scheie syndrome. Furthermore, the excreted acid mucopolysaccharide excretion in these cases are dermatan sulfate and heparan sulfate in urine, respectively. These excreted amount of acid mucopolysaccharides were also intermediate distribution between the above syndromes. $^{35}\text{SO}_4$ accumulation into acid mucopolysaccharide by H-S syndrome cells was similar levels as observed by those of Hurler and Scheie syndromes. The complementation by mixing Hurler cells with H-S cells using the parameter by $^{35}\text{SO}_4$ -acid mucopolysaccharide accumulation and degradation was not found, suggesting that a mutation site of defective enzyme is different each other.

- A48. マルチプル・サルファターゼ欠損症における遺伝生化学的研究：関口 茂** (埼玉県小児保健センター), **衛藤義勝** (慈恵医大・小児). **Genetical and Biochemical Study of Multiple Sulfatase Deficiency: Shigeru SEKIGUCHI** (Saitama Health Cent., Iwatsuki) and **Yoshikatsu ETO** (Dept. Pediat., Tokyo Jikei Univ. Sch. Med., Tokyo)

マルチプル・サルファターゼ欠損症は、アリルサルファターゼ A, B, C をはじめ少なくとも 7 つのサルファターゼが同時に欠損する先天性代謝異常症である。遺伝形式は常染色体劣性遺伝であるが、欠損酵素の 1 つであるアリルサルファターゼ C は X 染色体の Xg ローカスに存在する。

演者らは、マルチプル・サルファターゼ欠損症患児の母親の毛根のアリルサルファターゼ C 活性を測定した。活性値は正常からほぼ 0 まで分布し、モザイクズムがみられると考えられた。

- A49. 培養ヒトリンパ芽球における分枝鎖 α -ケト酸脱炭酸酵素：陣野吉広・赤星 泉** (熊本大・医・小児), **甲木孝人** (熊本大・医・微生物), **松田一郎** (熊本大・医・小児). **Branched-Chain Keto Acid Dehydrogenase Complex in Cultured Human Lymphoblasts: Y. JINNO, I. AKABOSHI** (Dept. Pediat., Kumamoto Univ.) **T. KATSUKI** (Dept. Microbiol., Kumamoto Univ.) and **I. MATSUDA** (Dept. Pediat., Kumamoto Univ., Kumamoto)

EB-ウイルスで感染・樹立したメープルシロップ尿症 (MSUD) 患者 5 例, 正常コントロール 3 例およびアメリカより手に入れた MSUD 細胞株 2 例の計 10 例を用いて, 分枝鎖 α -ケト酸脱炭酸酵素活性を測定した。また, 培地に分枝鎖アミノ酸を 10 mM ないしは 20 mM 添加して, 細胞増殖に及ぼす影響を調べた。酵素活性測定は, Fenson らの方法に従った。これにより以下の結果が得られた。1) 活性は正常コントロールの細胞では細胞増殖に同調して著明に増加し, 培地交換後 4~5 日で最高値を示した。このような酵素活性の増加は, 調べられた患者細胞株 3 例では認められなかった。2) 患者細胞株では, ロイシンを基質として用いた場合, classical type で正常コントロールの 7% 以下, variant type で 13~16% の値を示した。3) 三つの分枝鎖アミノ酸中, ロイシン添加による細胞増殖抑制が顕著で, この抑制効果は添加ロイシン濃度および酵素活性欠損の程度とよく関連した。

- A50. Evaluation of Heterozygote of the Patient with Dihydrobiopterin Synthetase Deficiency by Quantitative Analysis of Urinary Neopterin: Haruo SHINTAKU, Gen ISSHIKI** (Dept. Pediat., Osaka City Univ. Med. Sch., Osaka), **Yutaka HASE, Tsumeo TSURUHARA and Toshiaki OURA** (1st Div. Pediat., Children's Med. Cent. Osaka City, Osaka)

Tetrahydrobiopterin deficiency has been described as a form of hyperphenylalaninemia in which severe neurological symptoms develop despite early treatment with low phenylalanine diet. The biosynthesis of biopterin (Bi) from guanosine triphosphate involves multiple enzymatic steps and the patients with dihydrobiopterin synthetase (DHBS) deficiency have a block between dihydroneopterin triphosphate and dihydrobiopterin, which can be diagnosed by the quantitative analysis of unconjugated pteridines in serum and urine. We

have already reported that urinary neopterin (Ne) and Bi analysis was most important for diagnosis of DHBS deficiency and that Ne and Bi excretion in urine should be compared with the age matched control values because it changes remarkably with age. In this study we examined the excretion of urinary Ne and Bi in four parents of two patients with DHBS deficiency.

[Methods and Results] Analysis of urinary unconjugated pteridines was performed by a modification of the method described by Kohashi *et al.* using HPLC on ODS column. Urinary Ne and Bi excretions (nmol/mmol creat.) were as follows; 380 ± 244 , 300 ± 140 in healthy children (N=17), 130 ± 64 , 131 ± 36 in healthy adults (N=8), 275 ± 64 , 115 ± 14 in four parents, 9,400 and 15,600, 41 and 102 in two patients, respectively.

[Discussion] Quantitative analysis of urinary Ne and Bi of their two children of DHBS deficiency showed marked elevation of urinary Ne and significant decrease of urinary Bi. Urinary Ne excretion of four parents was two times of the control values. However no significant difference was observed in urinary Bi excretion. These results suggest that the DHBS activity of the parents might be about half of the control value, and that the urinary Ne assay is useful to detect heterozygote of DHBS deficiency.

A51. Gyrate Atrophy of Choroid and Retiva with Hyperornithinemia: Hiroki NAKA-BAYASHI, Momoko SAITO (Dept. Pediat., Nihon Univ., Tokyo), Takeshi SAKIYAMA, Misao OWADA, Teruo KITAGAWA, Mutuko HAYAKAWA, Takashi ISHIKAWA and Akira NAKAZIMA (Dept. Ophthalmol., Juntendo Univ. Sch. Med., Tokyo)

Two sibling cases of hyperornithinemia with gyrate atrophy were studied clinically and biochemically.

A 20-year-old Japanese man was admitted to the hospital complaining of poor night vision and myopia. An eye examination revealed narrow visual fields, posterior subcapsular cataracts. Laboratory tests were performed and hyperornithinemia and ornithinuria were recognized. A oral ornithine loading test resulted in a marked increase in plasma ornithine levels but returned to preloading level after 4 hr. Urinary creatinine excretion was also markedly reduced. Ornithine- δ -aminotransferase activity was detected in cultured skin fibroblasts but found to be low. His elder sister has the same complaint and was diagnosed as having gyrate atrophy of the retina.

Both patients did not respond to pyridoxine treatment. At the present time proline is being supplied to them orally but more time is needed to monitor their response.

- A52. Causal Relationship between Histidinemia and Mental Retardation:** Keiya TADA, Hiraku TATEDA, Shinichiro ARASHIMA, Kaoru SAKAI, Teruo KITAGAWA, Kikumaro AOKI, Seizo SUWA, Masahiko KAWAMURA, Toshiaki OURA, Masashi TAKESADA, Yasuhiro KURODA, Fumio YAMASHITA and Ichiro MATSUDA (A collaborative study group of neonatal screening for inborn errors of metabolism in Japan)

A nation-wide screening program among newborn infants for phenylketonuria, maple syrup urine disease, homocystinuria, histidinemia and galactosemia, using dried blood spots on filter paper and a microbiologic assay, was started in Japan in 1977. Among these disorders, the incidence of histidinemia was highest, 1 in about 8,000. However, the treatment of histidinemia remains controversial because of a lack of large-scale data.

The present study was undertaken to evaluate a causal relationship between histidinemia and mental retardation. Patients with untreated histidinemia were identified by survey of the siblings and parents of newborn infants with histidinemia found by a mass-screening program. Seventy-seven cases of untreated histidinemia, their ages being 3 to 45 years, were found. Developmental or intelligence quotients in 46 cases ranged from 82 to 143, the average being 105. The remaining 31 patients, in whom IQ was not assessed, were considered to be mentally normal from the interview and from professional or school records. Twelve children born to histidinemic mothers also were found in this survey. These children were physically and mentally normal.

These findings suggest that histidinemia is a harmless disorder as far as mental retardation is concerned.

- A53. Beta-Galactosidase in Twitcher Mice (Mice Krabbe's Disease):** Yoshikatsu ETO, Fusayo UMEZAWA and Etsuko KASAI (Dept. Pediat., Tokyo Jikei Univ. Sch. Med., Tokyo)

The twitcher mouse has recently been identified as a model for human globoid cell leukodystrophy. The disease transmitted by an autosomal recessive gene (*twi*). It is characterized by severe demyelination in peripheral nerves and the presence of globoid cells. Biochemically, the deficiency of galactocerebroside beta-galactosidase was also found in various organs of twitcher mice. These pathological and biochemical findings in twitcher mice closely resemble those found in human globoid cell leukodystrophy. In present report, beta-galactosidase in twitcher mice tissues are characterized. 4-MU beta-galactosidase activity in various tissues were within normal range. HNP-galactosidase activity was not deficient in twitcher mice tissues, whereas human Krabbe's disease' tissues contained less than 10% of control activity. To detect homozygote by the determination of HNP-galactosidase activity in mouse tail failed to determine. pH activity profile of cerebroside

beta-galactosidase in twitcher mice brain showed pH 4.5 as an optimum, which are similar to that of control.

The drastic deficiency of cerebroside beta-galactosidase in twitcher tissues resulted in an accumulation of galactocerebroside in globoid cells and also in kidney.

A54. アカタラセミアマウス肝カタラーゼの等電点電気泳動による分析：佐藤征紀・緒方正名（岡山大・公衆衛生）. Analysis on Liver Catalase of Acatalasemic Mice by Isoelectric Focusing: Yukinori SATO, Masana OGATA (Dept. Public Health, Okayama Univ., Okayama)

アカタラセミアマウスのカタラーゼ分子は正常とは構造上の変異があるとされているが、直接的に分析を等電点電気泳動で行った。正常、アカタラセミア同型接合体、ヒポカタラセミア同型接合体、アカタラセミア異型接合体各 3 匹より肝を分取し、肝ホモジネートを $105,000 \times g$ の遠心による細胞分画法で肝カタラーゼ酵素上清液を用意し、 6°C , pH 2~12 のアガロース等電点電気泳動法で分離し、KI 染色を施した。その結果、pH 4.9 付近の細いバンドと pH 7.5~5.3 にわたる幅広いバンドの 2 種が認められたが、広いバンドの中心点の pH の平均値と比較すると、アカタラセミアマウス肝のカタラーゼの等電点はアルカリに近く、正常マウス肝のカタラーゼは酸性側に近くバンドが形成される傾向があった。また、ヒポカタラセミアおよびアカタラセミア異型接合体は比較的正常に近いバンドを示した。

このことから、アカタラセミアの肝カタラーゼ分子の等電点は正常のそれよりもアルカリ側にあることから、両者の蛋白質の構造に差異があると推定される。

A55. Analysis of Specific Proteins in Patients with Systemic Lupus Erythematosus: Fumitaka MORITO, Akihide OHTA, Toshiro NAGAYOSHI and Masaya YAMAGUCHI (Dept. Intern. Med., Saga Med. Sch., Saga)

The development of Systemic Lupus Erythematosus (SLE) is suspected to be concerned with the genetic factor which is susceptible to multifactorial inheritance most likely. The variant genes which have submajor effects on the development of SLE may produce some particular substance.

We analyzed the protein components of lymphocytes and sera from SLE patients, their relatives, and normal controls by two-dimensional gel electrophoresis. Comparisons by all these polypeptides patterns revealed that three main variant spots might differ between SLE patients and normal controls. Among the three variant polypeptides, two variant spots in the PHA-stimulated lymphocyte proteins were with mol. wt. 70,000/PI 7.0 (Lp. 1) and mol. wt. 41,000/PI 6.0 (Lp. 17). The variant polypeptide in the serum protein was with mol. wt. 31,000/PI 5.8 (S. 1).

These three SLE specific spots were present in 70–80% of SLE patients, and in 20–40% of normal controls. The frequencies of possessing these SLE specific proteins in the relatives were in the intermediate range between SLE group and normal control group. Much

of SLE patients (56%) have all of these three SLE specific proteins, but rarely normal controls.

These data suggest that these variant polypeptide spots might be concerned with the genetic factors contributed to the development of SLE.

A56. Biochemical Studies on Amyloid Fibril Proteins: Tomotaka SHINODA, Fuyuki KAMETANI, Satoru SUZUKI (Dept. Chem., Tokyo Metropol. Univ., Tokyo), Shozo KITO and Masae INOKAWA (Dept. Med., Hiroshima Univ., Hiroshima)

Amyloid fibril proteins were extracted with H₂O using autopsy samples from individuals with Ogawa Village type familial amyloidosis and were purified by gel filtration with Sephadex G-75/100 followed by ion exchange column chromatography with Sephadex A-50 in 8 M urea using the samples solubilized with 6 M guanidine hydrochloride at an alkaline condition. Five major components have thus far been obtained. Their molecular weights were estimated by SDS-disc electrophoresis to be 16,000, 25,000, 35,000, 50,000 and 70,000. On immunodiffusion they were shown to have no cross reactivities against specific antisera to normal human serum proteins except for prealbumin. Isoelectric points of these proteins were similar to each other and were estimated to be 4.8. Amino acid compositions were also similar to each other and had the following characteristic (residue %): Lys, 6.0; His, 2.7; Arg, 4.4; Asp, 8.5; Thr, 7.2; Ser, 7.3; Glu, 14.4; Pro, 6.0; Gly, 6.0; Ala, 9.3; Cys/2, 0.4; Val, 7.2; Met, 1.2; Ile, 3.2; Leu, 6.2; Tyr, 3.6; Phe, 4.4. The composition were rather similar to that of amyloid proteins isolated from individuals with Portuguese type familial amyloidosis, but were different from that of prealbumin in contrast to the evidence that the proteins had apparent cross reactivities against antiprealbumin antiserum. Preliminary results for amino terminal analysis indicated that they had glycine as the terminal residue.

A57. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. 3. Genetic Polymorphism of Proteins with pI Less than 7: Ikuko KONDO, Michiko YAMADA, Kimiko YAMAKAWA (Div. Hum. Genet., Univ. Tsukuba), Masanao SHIBASAKI (Div. Pediat., Univ. Tsukuba) and Hideo HAMAGUCHI (Div. Hum. Genet., Univ. Tsukuba, Ibaraki)

Leukocytes derived from 2 ml of peripheral whole blood were cultured with RPMI 1640 medium containing PHA and 10% FCS for 6 days, and 30 μ g of protein in supernatants after centrifugation at 105,000 $\times g$ for 1 hr was loaded on 1st dimensional gels. Two-dimensional gel electrophoresis was performed according to O'Farrell (1975) with minor modification: Mixtures of 1.4% Ampholine pH 5-8 and 0.4% Ampholine pH 3.5-10 were used. About 250 polypeptide spots have been identified in gels stained with silver staining

method (Merril *et al.*, 1981). The pH distribution was linear, ranging from 8 to 5 in the first dimension. Common variants were observed in 6 polypeptides with mol. wt. of 100,000, 64,000, 49,000, 40,000, 37,000 and 30,000. Family study indicated that each polypeptide is determined by a single autosomal locus, and in a Japanese population, the gene frequencies of these polypeptides were 0.912 and 0.088 in 100k polypeptide, 0.681 and 0.319 in 49k polypeptide, 0.532 and 0.468 in 40k polypeptide, 0.881 and 0.119 in 37k polypeptide, 0.927 and 0.073 in 30k polypeptide, and 0.936 and 0.064 in 64k polypeptide.

A58. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. 4. Genetic Polymorphism of Proteins with *pI* More than 7: Michiko YAMADA, Ikuko KONDO, Kimiko YAMAKAWA (Div. Hum. Genet., Univ. Tsukuba), Masanao SHIBASAKI (Div. Pediat., Univ. Tsukuba) and Hideo HAMAGUCHI (Div. Hum. Genet., Univ. Tsukuba, Ibaraki)

Genetic analysis of approximately 250 PHA-stimulated peripheral blood lymphocyte polypeptides with *pI* more than 7 was carried out by nonequilibrium pH gradient electrophoresis (O'Farrell *et al.*, 1977). Polypeptides were visualized on the gel by silver stain (Merril *et al.*, 1981). Three phenotypes were observed in each of two polypeptides with mol. wt. of 68,000 (68k polypeptide) and with mol. wt. of 44,000 (44k polypeptide). Family and population studies indicate that the three phenotypes of each of the two polypeptides are determined by two common alleles at a single autosomal locus. In a Japanese population, the gene frequencies of the two alleles of 68k polypeptide were 0.65 and 0.35, and those of 44k polypeptides were 0.75 and 0.25, respectively.

A59. Genetic Analysis of Human Lymphocyte Proteins by Two-Dimensional Gel Electrophoresis. 5. Some Properties of Polymorphic Cellular Abundant Proteins: Hideo HAMAGUCHI, Kimiko YAMAKAWA, Michiko YAMADA (Div. Hum. Genet., Univ. Tsukuba), Atsuo NOGUCHI (Div. Immunol., Univ. Tsukuba) and Ikuko KONDO (Div. Hum. Genet., Univ. Tsukuba, Ibaraki)

We have reported that a genetic polymorphism determined by a single autosomal locus is present in each of 8 cellular polypeptides, in contrast to the previous concept of the extremely restricted genetic variability of cellular abundant polypeptides. As the first step of the experiments for the elucidation of the properties of the polymorphic cellular abundant polypeptides, we examined the cellular localization and the polymeric state of them. Cytosol and microsomal fractions were obtained from 2 human B-lymphoblastoid cell lines, Rajii and OV1, by the method of Littlefield and Keller (1957). Cytosol proteins were further fractionated on Sephadex G-200 column, 1.6×90 cm, using 4 mM MgCl₂, 25 mM KCl, and 50 mM Tris buffer, pH 7.6. Each fraction was analyzed by O'Farrell's two di-

mensional gel electrophoresis (1975). It is observed that 7 polymorphic polypeptides, 100k, 68k, 64k, 49k, 40k, 37k, and 30k polypeptides are present in the cytosol. On the other hand, 44k polypeptides seems to exist in membranes. Two-dimensional gel electrophoresis patterns of fractionated proteins indicated that 64k and 30k polypeptides are present as monomer, and that 100k, 49k and 37k polypeptides as homo- or hetero-oligomer. The polymeric state of 68k and 40k polypeptides are not determined yet. The data suggest that most of polymorphic cellular abundant polypeptides exist as monomer or oligomer in the cytosol.

A60. Mitochondria DNA Polymorphism—Identification with Ethidium Bromide:

Shoji HARADA (Dept. Community Med., Univ. Tsukuba, Ibaraki)

The maternal inheritance of human mitochondrial DNA was demonstrated by Case and Wallance (1981) using restriction endonuclease cleavage pattern of the DNA. Normally, DNA fragments after agarose gel electrophoresis are identified by Southern gel hybridization procedure or ethidium bromide staining method. The former has the high specificity and sensitivity more than the method of ethidium bromide. However, experimental procedure is very complicated. The latter is simple and rapid, if DNA is perfectly purified.

In the present study, the simple and rapid method to purify DNA from human placenta mitochondria was improved using agarose gel electrophoresis. The original methods described by Yang and Wu (1979) was modified. The slice of the agarose gel containing DNA isofragment after electrophoresis is dissolved in 4 volumes of 6 M NaClO₄ at 50–60°C for few min. DNA is stained by ethidium bromide. The DNA in the dissolved agarose adheres to a single 10 mm-diameter glass filter (Whatman GF/C) which are placed on a single-glass filtration set under water aspiration. After that, the glass filter is washed with 1 ml of NaClO₄-Tris buffer and then 1 ml of isopropanol and 1 ml of ethanol and then, dried in the room temperature for few min using hair dryer. The DNA on the glass filter is eluted by reaction buffer of restriction endonuclease. The elution buffer of 100 μ l is added to the filter containing DNA in the Eppendorf microtube. The bottom of tube is pierced with needle and then eluate is collected by 12,000 $\times g$ for 10 sec. When 30 g of placenta is used, ca. 10 μ g of purified DNA was obtained in the present method. Eco-R1, Hind-III and Hha-I were used in the present study as restriction endonuclease. In this study, the sample size was too small to find variants of DNA fragments.

A61. Long Terminal Repeat-Like Elements Flank A Human Immunoglobulin Epsilon Pseudogene That Lacks Introns: Shintaro UEDA (Dept. Anthropol., Fac. Sci., Univ. Tokyo, Tokyo), Sumiko NAKAI, Yasuyoshi NISHIDA, Hiroshi HISAJIMA and Tasuku HONJO (Dept. Genet., Osaka Univ. Med. Sch., Osaka)

There are at least three immunoglobulin epsilon genes ($C\epsilon_1$, $C\epsilon_2$ and $C\epsilon_3$) in the human genome. The nucleotide sequences of the expressed epsilon gene ($C\epsilon_1$) and one ($C\epsilon_3$) of the two epsilon pseudogenes were compared. The results show that the $C\epsilon_3$ gene lacks the three intervening sequences entirely and has a 31-base A-rich sequence 16 bases 3' to the putative poly (A) additional signal, indicating that the $C\epsilon_3$ gene is a processed gene. The $C\epsilon_3$ gene sequence is homologous to the five separate DNA segments of the $C\epsilon_1$ gene; namely, a segment in the 5' flanking region (100 bases) and four exons, which are interrupted by a spacer region or intervening sequences. Long terminal repeat (LTR)-like sequences which contain TATAAA and AATAAA sequences as well as terminal inverted repeats are present in both 5' and 3' flanking regions. The 5' and 3' LTR-like sequences do not, however, constitute a direct repeat, unlike transposable elements of eukaryotes and retroviruses. The 3' LTR-like sequence is repetitive in the human genome, but is not homologous to the Alu family DNA. Models for the evolutionary origin of the processed gene flanked by the LTR-like sequences are discussed. The $C\epsilon_3$ gene has a new open frame which codes potentially for an unknown protein of 292 amino acid residues (Ueda *et al.*, *EMBO J.*, in press).

B1. Bloom Syndrome B- and T-Cell Lines Transformed with Epstein-Barr- and Adult T-Cell Leukemia-Virus: Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch.), Isao MIYOSHI (Dept. Med., Kochi Med. Sch., Nankoku), Naomi KONDO and Tadao ORII (Dept. Pediat., Gifu Univ., Gifu)

We have established both B- and T-cell lines from the peripheral blood of two Bloom syndrome (BS) patients and one healthy female by using Epstein-Barr- (EB) and Adult T-cell leukemia- (ATL) virus. The cell lines from normal and BS subjects exhibited cell surface markers compatible with B- and T-cell origin, and BS B- and T-cell lines retained the original cytogenetical characteristics of the syndrome. Even though PHA stimulated BS lymphocytes from two BS patients studied showed the original high levels of sister chromatid exchanges (SCEs), the BS B-lines established with EBV yielded two separate lines each, *i.e.*, one with increased SCE and another with normal levels of SCE, also one of the BS T-lines retained the high SCE level in 100% of the cells and the other BS T-lines contained two populations, one with high SCE (70%), and the other with normal (30%), at a relatively constant frequency over 6 months. Neither EBV nor ATLTV caused a significant increase in chromosome instability in established lines compared to fresh lymphocytes. These results strongly suggest that BS patients have two populations *in vivo*, one with high and other with normal level of SCE at least in the lymphoid cell system. EBV and ATLTV infection, chromosome instability, karyotypic abnormality and SCE in BS B- and T-lymphocytes were also discussed.

B2. Effect of Inhibitors of Poly (ADP-Ribose) Polymerase on SCE Frequency in Bloom Syndrome (BS) Cells: Yukimasa SHIRAISHI (Dept. Anat., Kochi Med. Sch., Nankoku) and Masanao MIWA (Natl. Cancer Cent. Res. Inst., Tokyo)

Recent studies have shown that benzamide (Bam) and m-aminobenzamide (m-AB), two potent inhibitors of poly(ADP-ribose) polymerase, increase the frequencies of SCEs in human and Chinese hamster cells in a dose-dependent manner. It should be valuable to examine the effect of Bam and m-AB on SCE frequencies and poly(ADP-ribose) metabolism in BS-B lymphoid cells, which are characterized by a spontaneously high incidence of SCE.

Results are presented that Bam (5 mM) and m-AB (10 mM) increased the SCE to the levels of 87.8 (Bam) and 90.4 (m-AB) SCE per cell, respectively, as compared with that of control level (73.4 SCE per cell). The value of increase of SCE in BS cells in almost the same degree to that in normal cells treated with these inhibitors. The significance of poly (ADP-ribose) metabolism in high SCE level in BS cells will be discussed.

B3. Effects of Cell Fusion on SCE Levels of Bloom Syndrome and Normal B-Lymphoid Cells: Yukimasa SHIRAISHI and Takahiro TAGUCHI (Dept. Anat., Kochi Med. Sch., Nankoku)

The effects of cell fusion on SCE levels in BrdU labeled Bloom syndrome (BS) or normal cells, have been studied. When normal cells labeled with BrdU for one round of DNA replication were fused with nonlabeled BS cells, the SCE frequency of the normal chromosomes in the hybrid cells increased strikingly to levels of 30–40 SCE per cell. On the other hand, when BS cells labeled with BrdU were fused with nonlabeled normal cells the BS chromosomes had a normal level of SCE at first mitosis after fusion.

These findings strongly indicate that SCE regulatory factors (SCE-normalizing and SCE-causing factors) are present in normal and BS-cells, their expressions and/or concentrations possibly being related to the nature of the abnormal state.

B4. Contribution of 5-Bromodeoxyuridine to the Frequency of Spontaneous Sister-Chromatid Exchanges in Bloom's Syndrome Fibroblasts: Takayuki KURIHARA (Cent. Lab., Kanazawa Med. Univ.), Kouichi TATSUMI (Rad. Biol. Cent., Kyoto Univ., Kyoto) and Masao INOUE (Cent. Lab., Kanazawa Med. Univ., Ishikawa)

Cells from patients with Bloom's syndrome (BS) exhibit extremely high frequency of spontaneous sister-chromatid exchanges (SCEs). However, the nature of cytogenetic phenomenon in BS cells is unknown. Generally, it is necessary to incorporate 5-bromodeoxyuridine (BUdR) into chromosome during the first cell cycle or two cell cycles, in order to be able to score SCEs. However, the frequency of SCEs is dependent on BUdR concentration in medium, since BUdR itself induces SCEs. We attempted to determine the frequency of SCEs in BS cells grown in medium containing the various concentration of BUdR for two and three cell cycles. In the second-division chromosomes, the increased rate of SCEs in BS cells was 1.5~8.5-fold larger than that found in normal cells at concentration of 1–5 μM , but there was no significant difference between BS and normal cells for the increased rate at concentration of 10 μM or higher. In the third-division chromosomes, the SCE nonreciprocal/reciprocal ratios of BS cells grown in all three replication periods in the presence of BUdR were 3.0 : 1 at concentration of 5 μM and 2.9 : 1 at 10 μM , and its ratios of BS cells grown in only the first replication period in its presence were 4.4 : 1 at 5 μM and 3.5 : 1 at 10 μM , provided that those detected as "reciprocal" are formed during the last cycle, while those appearing as "nonreciprocal" have been produced during either the first or the second cycle (J.B. Schwartzman *et al.*, *Genetics*, 1979).

These results suggest that high frequency of spontaneous SCEs in BS cells may depend on BUdR concentration and timing incorporating BUdR into cellular DNA.

B5. Sister Chromatid Exchanges in Early Mouse Embryos *in Utero*: Sumio IJIMA, Makoto HIGURASHI (Dept. Health Sci., Yamanashi Med. Univ., Yamanashi), Kanehisa MORIMOTO, Mayumi SATO and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

Control and Mitomycin C (MMC, 10 mg/kg)-treated pregnant mice were infused intravenously with 160 $\mu\text{g/g/hr}$ of 5-bromodeoxyuridine (BrdU) for 22.5 hr on day 8 of gestation. Maternal bone marrow, embryos and yolk sacs were harvested. Sister chromatid differentiation was done by fluorescent plus Giemsa technique.

Embryos and yolk sacs had baseline SCE frequencies of 4.6 and 6.6 per cell, respectively, maternal bone marrow having a frequency of 10.0 per cell. Treatment with MMC resulted in dose-related increases in SCE frequencies in these three tissues: For example, 64.8 in bone marrow, 48.2 in embryos and 56.6 in yolk sacs when mice were administered with 10 mg/kg MMC.

The relative sensitivities of embryo and yolk sac to maternal bone marrow were calculated; such values were 1.6 in embryo and 1.3 in yolk sac. These data were compared with those of Kram *et al.* (1979) which showed 1.0–1.1 relative sensitivities on day 13 embryos. This suggest that early embryos may be more sensitive than later embryos to mutagens.

B6. Further Lymphocyte Chromosome Survey among 23 Patients with Retinoblastoma Using More Extensive Methods: Tomiko MOTEGI, Makiko KAGA, Hiroko KADOWAKI, Akira INOUE, Mitsumasa SHIMIZU (Dept. Pediat., Tokyo Univ. Branch Hosp.), Mari KOMATSU and Kensei MINODA (Dept. Ophthalmol., Tokyo Univ. Branch Hosp., Tokyo)

We had found two cases with a 13q14 deletion mosaicism in lymphocytes among 42 retinoblastoma patients with an effort to detect it in the previous study (1980). Now further twenty-three retinoblastoma patients (Patient Nos. 44 to 66) were examined using more extensive methods in order to ascertain the role of 13q14 deletion mosaicism in the etiology of this tumor. Sixteen patients had bilateral retinoblastoma (11 sporadic and 5 familial) and 7 unilateral (4 sporadic and 3 familial). Using a cell population with a high proportion of early mitotic cells with ethidium bromide pretreatment 2 hr before harvest according to the methods described by Ikeuchi and Sasaki (1979) and by examining more than 100 cells, we found further three cases with a 13q14 deletion mosaicism. These three patients had sporadic retinoblastoma, of which two had bilateral (Patient Nos. 44 and 45) and one unilateral (No. 54). The results indicate that a 13q14 deletion mosaicism plays a substantial role in the etiology of this tumor.

Two other patients had different chromosomal aberrations. These are a monosomy 13(q12.3-q21.31) (Patient No. 50) and a *de novo* extra small submetacentric marker chro-

mosome in mosaicism (Patient No. 59). The salient clinical features in the patient with a monosomy 13(q12.3-q21.31) were a tetralogy of Fallot, severe psychomotor retardation, muscular hypotonia, facial dysmorphism and abnormally postured feet besides sporadic bilateral retinoblastoma. Another patient with an extra marker chromosome had familial unilateral retinoblastoma, whose father and brother had had this tumor. The fact that the marker chromosome was not found in either her parents or her brother suggests the negative relationship of the cytogenetic finding to retinoblastoma.

B7. Assignment of the Genes for Wilms Tumor-Aniridia, Genitourinary Abnormalities, and Mental Retardation Triad (WAGR) and Catalase (CAT) on the Middle Portion of the 11p13 Band (Possibly p1305→p1306): K. NARAHARA, K. KIKKAWA, S. KIMURA (Dept. Pediat., Okayama Univ.), M. OGATA (Dept. Public Health, Okayama Univ.), R. KASAI (Asahigawa Jidoin Children's Hosp., Okayama), M. HAMAWAKI (Dept. Pediat., Kochi Municipal Cent. Hosp.), K. MATSUOKA (Dept. Surg., Kochi Municipal Cent. Hosp., Kochi) and H. KIMOTO (Dept. Pediat., Okayama Univ., Okayama)

An interstitial deletion of 11p13 is known to be the cause of WAGR. The structural gene for the red blood cell CAT has recently been assigned to 11p13 (Junien *et al.*, 1980). The accurate locus for WAGR and CAT on 11p13, however, is unknown. We studied the red blood cell CAT levels in two unrelated cases of interstitial deletions of 11p, and tried to determine the accurate locus of those genes.

Case 1 was a boy, aged 3 years and 7 months. The child showed aniridia, bilateral Wilms tumor, short stature, mental retardation and ear malformations. Genital abnormalities were not present. He died from renal failure at 3 years and 8 months. Case 2 was a mentally retarded 18-year-old boy. He had aniridia, ear malformations, cryptorchidism and short stature. Extensive examinations disclosed no neoplasms in the genitourinary system.

Chromosome analyses were performed on peripheral lymphocytes using the high resolution banding techniques. The karyotypes of Case 1 and 2 were 46,XY,del(11)(pter→p13::p11.11→qter) and 46,XY,t(2;17)(q23;q25), del(11)(pter→p13::p11.3→qter), respectively. The distal breakpoints of 11p in both cases appeared to have occurred on the middle portion of 11p13 (possibly 11p1305→p1306). The red blood cell LDH isoenzyme patterns in the two cases were consistent with hemizygous deficiency of LDH-A, which was mapped to 11p12. The red blood cell CAT levels were measured with the method of Matsubara *et al.* (1969). Case 1 had a 50% CAT level of normal persons and was interpreted as hemizygote for CAT gene, while Case 2 had a low normal CAT level (80% of normal value).

Previous studies have demonstrated the crucial role of the distal half of 11p13 in pro-

ducing WAGR phenotype (Riccardi *et al.*, 1978; Francke *et al.*, 1979). The findings in our cases tend to indicate that both WAGR and CAT locus are on the middle portion of 11p13 (possibly 11p1305→p1306), and in that region CAT is more distally placed than WAGR.

B8. Fibroblasts from Tuberous Sclerosis Patients; Sensitivity to DNA Damaging Chemicals and Growth Properties: Kousaku OHNO and Kenzo TAKESHITA
(Div. Child Neurol., Tottori Univ., Yonago)

Tuberous sclerosis (TS) is an autosomal dominant disease characterized by epilepsy, mental retardation, and angiofibroma of the face. To search the expression of the gene in cultured skin fibroblasts, we have studied several *in vitro* properties of fibroblasts from the patients with TS. Fibroblasts from 5 patients (24.8 ± 7.7 years old) and 5 controls (25.2 ± 10.8 years old) were studied for the colony forming ability of cells after incubation in medium containing various doses of MNNG, EMS, MMC, and 4NQO for 24 hr, and for the growth curve analysis of cells cultured in medium containing 10% fetal bovine serum at 37°C. In preliminary experiments we confirmed that the sensitivities of cells from different aged donors to the drugs, except for 4NQO (this was not examined), were almost the same, and that the sensitivities of cells at different growth states (such as exponentially growing, subconfluent, or confluent resting state) to the drugs were considerably different, except for EMS. In an experiment used subconfluent cells of 2 TS strains and 4 control strains, the different sensitivities to MNNG, EMS, or MMC between two groups were not detected. In second experiment used cells cultured for 48 hr after subculturing the subconfluent cells from 5 TS strains and 5 control strains, the results showed that cells from TS had the same sensitivities to EMS and MMC as controls, but cells from TS had slight increased sensitivity to MNNG and slight decreased sensitivity to 4NQO. The different sensitivities to MNNG and 4NQO were considered to be able to attribute that some of TS strains had contained more exponentially growing cells during exposure to the drugs than controls, because the growth curve analysis using the same cells as used in the survival assay showed that lag times until exponential growth of several TS strains were shorter than those of controls. The growth rates and the saturation densities of 5 TS strains were almost the same as those of controls.

B9. Cytogenetic and Cytokinetic Analysis of Lymphocytes from Patients with Hereditary Adenomatosis of the Colon and Rectum: Toshihiko KASUKAWA, Toshiaki WATANABE and Akira ENDO (Dept. Hyg. Prev. Med., Yamagata Univ. Sch. Med., Yamagata)

The frequencies of chromosome aberrations and sister chromatid exchanges (SCEs), and cell cycle kinetics were examined in cultured lymphocytes from 5 patients with hereditary

adenomatosis of the colon and rectum (ACR). Three patients were with Gardner's syndrome (GS) and two patients (mother and her daughter) with familial polyposis coli (FPC). Chromosome preparations were made by the conventional air-drying method and sister chromatids were differentially stained by a modified FPG method.

The frequency of numerical chromosome aberrations was not different in the metaphase cells in both first and second replication cycles (M1 and M2) between ACR patients and control subjects. However, the percentage of structural chromosome aberrations in both M1 and M2 cells was slightly higher in ACR patients as compared with controls.

Neither spontaneous nor MMC-induced SCE frequencies in the patients with ACR were different from the controls except for one patient with GS, who remarkably higher spontaneous SCE frequency. This patient was a mother of a son who got hepatoblastoma, a case similar to those reported by Kingston *et al.* (*Lancet* I, 457, 1982). She also has a goiter.

The cell replication index was lower in GS patients than in FPC patients and controls, showing *in vitro* delay of cell proliferation.

Results of 2q heteromorphism analysis in ACR patients were equivocal as yet and we could not confirm the findings of a recent report by Gardner *et al.* (*Cancer* 49, 1413-1419, 1982).

B10. 大腸腺腫症における遺伝的異質性の検討：谷村雅子・宇都宮譲二（東医歯大・ポリポーシスセンター）. Genetic Heterogeneity in Adenomatosis Coli: M. TANI-MURA, J. UTSUNOMIYA (Poliposis Cent., Tokyo Med. Dent. Univ., Tokyo)

大腸腺腫症におけるガードナー徴候 (GS) の有無とポリープ密度型に関する関係と遺伝的異質性を、登録例 412 家系 608 例を用いて調べた。ポリープ密度型が発端者と異なる罹患家系員は 9/53 であったが、判定精度を考慮すると遺伝的に密生型か非密生型に決まる可能性が大きい。GS である体表の骨腫 (H) と軟部組織腫瘍 (S) との出現に、 $r=0.37$ の有意な相関性が認められた。罹患家系員の GS 出現率は発端者 H(+) の場合も S(+) の場合も H, S とも高く、H(+) または S(+) 率は発端者 H(-)S(-) では 18.9% に対し発端者 H(+) または S(+) では 38.1% と高いが、発端者 H(+)S(+), H(+)S(-), H(-)S(+) 群間では差が見られなかった。発端者 GS(+) の親または子、同胞、その他の 3 群間にも差はなかった。したがって、S と H との出現に共通の要因が働いており、多因子ではなく主に単一の遺伝因子に支配されている可能性が強い。H(+) 率も S(+) 率も、密生型 (23.1%) より非密生型 (37.6%) に多かった。罹患家系員 GS(+) 率は非密生家系で発端者 GS(+) の家系に最も高く (41.2%)、発端者 GS(-) でも密生型より非密生家系に高かった。他の随伴病変の出現に関し、GS(+)・非密生に GS(+)・密生と GS(-)・非密生が類似し、GS(-)・密生は類似性が低く、発端者の親の死因も同様であった。

以上より、本症に GS(-)・密生型と GS(+)・非密生型とがあり遺伝的に支配されている。また、GS(+)・非密生であるが不浸透のため GS(-)・非密生が、判定ミスのため GS(-)・密生の多くがあると考えるのが妥当であろう。

- B11. 大腸腺腫症における癌高危険度群対策の成績：大用幸世・谷村雅子・岩間毅夫**
 (東医歯大・ポリポージスセンター), 宇都宮讓二 (東医歯大・二外・ポリポージス
 センター). **Result of Cancer High Risk Group Approach for Family with Adeno-**
matosis Coli: Sachiyo DAIYO, Masako TANIMURA, Takeo IWAMA (Polyposis
 Cent., Tokyo Med. Dent. Univ.), Joji UTSUNOMIYA (2nd Dept. Surg. Polyposis
 Cent., Tokyo Med. Dent. Univ., Tokyo)

癌高危険度群対策の一環として遺伝疫学的アプローチが考えられるが、家系員の同調性が不明なため組織的に行われたことはなかった。大腸腺腫症においては早期発見法、治療法が存在するので、私どもは積極的アプローチを行うべきと考え、10 年来自験例の家族に説得により検査、治療の勧めを行ってきた。この経験を基にアプローチ方法・内容・時期・配慮事項・資料整理法等を検討し、全国からの登録例の家系員に対しても当センターからの組織的アプローチを試みた。

自験例においては 40 家系 195 名にアプローチを行い、70% から反応があり、うち 89% が検査希望、11% が検査拒否であった。検査は 115 名に行われ、42 名の罹患者が発見され 32 名が手術を受けた。登録例家系では、360 通の検査の勧めの手紙に対し 94 通 (26%) の返事があり、55% が検査希望であった。反応率が比較的良好であったので、今後も続けてゆく必要があると判断した。実行にあたっては、個人秘の厳守、患者の立場に立つ等に配慮すべきで、さらに通知の手段としては面接が良いことが受検査率 (77%) の結果から明らかになった。また、この説得方法と並行して郵送により検査を行うヘモカルト便潜血テスト法を試みた結果、検査拒否者、それ以前には反応のなかった者の 34% から返送があり、大腸検査へのきっかけをつかむには本法は有用であることがわかった。

以上、悪性腫瘍性疾患に対して遺伝疫学的アプローチが可能であり有効であることが判明した。

- B12. An Epidemiologic Study on Familial Predisposition to Large Bowel Cancer:**
Motoi MURATA (Nat'l. Inst. Radiol. Sci., Chiba) and **Takashi TAKAHASHI**
 (Cancer Inst. Hosp., Tokyo)

In order to investigate hereditary factor in large bowel cancer, it was attempted to clarify possible clinico-epidemiological characteristics of a familially predisposed patient. Hospital records of 940 male and 689 female patients, excluding those of familial polyposis, in the Cancer Institute Hospital, Tokyo, in 1946-1979 were utilized. They were divided into several groups by sites and multiplicity of the tumor. Family history of cancer was confined to those in the first and second degree relatives. Totally, 98 patients (6%) were recorded to have one or more relatives with colo-rectal cancer, who will be called a familial patient. Frequency of familial patients was highest in those with asynchronous multiple cancers (31%) and lowest with rectum cancer (4%). Average age at surgery was 55.0 years in familial patients, which was only slightly younger than that (56.8 years) of non-familial patients. If confined to those with proximal colon and multiple cancers, however, age reduction due to the positive family history was remarkable and statistically significant. Frequency of cases with polyp was 24% in total patients and did not show any significant difference with and without a positive family history of cancer. It may be concluded that

the large bowel cancer in the familiarly predisposed is characterized with early age at onset and tendency for multiplicity or for site of proximal colon. Such kind of patients would probably be genetically predisposed, so that they should be engaged for further genetic investigations.

B13. Cytogenetic Studies of Benign Cystic Teratomas of the Ovary: Kazushi NO-MURA, Etsuji OKAMOTO, Koso OHAMA, Atsushi FUJIWARA (Dept. Obst. Gynec., Hiroshima Univ.) and Yasuhiko FUKUDA (Dept. 2nd Surg., Hiroshima Univ., Hiroshima)

In order to investigate the origin of gonadal teratoma, sixteen benign cystic teratomas (dermoid cysts) of the ovary obtained from four patients were studied by using some genetic markers such as chromosomal Q- and R-heteromorphisms, histocompatibility leucocyte antigen specificities and phosphoglucutase-1 and esterase D genotypes.

Of sixteen dermoid cysts analyzed, thirteen showed homozygous pattern of chromosomal heteromorphisms which was one of the two homologous chromosomes of their hosts. In four of these tumors, either HLA antigen specificities or enzyme genotypes showed heterozygous pattern, and in nine tumors, all the genetic markers analyzed were homozygous. The above findings suggest that these thirteen tumors were arisen from a germ cell after the first meiosis. The likely mechanism is the suppression of the second meiotic division or the fusion of second polar body with the oocyte. On the other hand, heterozygous pattern of chromosomal heteromorphisms was observed in the remaining three tumors. In two of them, HLA antigen specificities and/or enzyme genotypes were homozygous, while their host cells showed heterozygous pattern. These findings strongly suggest that these three tumors were originated from a germ cell before the first meiosis.

In conclusion: 1) benign cystic teratomas of the ovary originate from a germ cell not only after the first meiosis but also before the first meiosis, 2) tumors occurred through different processes are present in one ovary.

B14. ヒト卵巣類内膜癌由来細胞株の染色体分析: 荘 漢一・上原茂樹・阿部洋一・小沢信義・佐藤信二・矢嶋 聡・鈴木雅洲 (東北大・医・産婦人). Karyotypical Analysis of an Human Ovarian Endometrioid Carcinoma Cell Line: Kanichi SOH, Shigeki UEHARA, Yoichi ABE, Nobuyoshi OZAWA, Shinji SATO, Akira YAJIMA, Masakuni SUZUKI (Dept. Obst. Gynec., Tohoku Univ., Sendai)

最近、われわれは、卵巣の類内膜癌患者の腹水から得た癌細胞を 37°C, 5% CO₂ 下で 15% FBS 添加 Eagle's MEM を培地として継代培養し、細胞株を樹立した。癌細胞の colony は敷石状に配列する類円形の細胞によって形成され、重積傾向もみられ、細胞の倍加時間は約 48 時間であった。継代されてきた細胞、約 10⁷ 個をヌードマウス背部皮下に移植し、2 カ月後には直径 1.5 cm ほどに発達した移植腫瘍が得られた。その病理組織像は未分化型腺癌と思われる所見で、原病巣とは異なった

ものとなった。

細胞株を経時的に、その染色体構成を分析した。継代 5 代目に施行した染色体分析では染色体数は 47 にモードを有し、かなり狭い範囲に分布する hyperdiploid type であった。1 本の分類不能の submetacentric なマーカー染色体が、ほとんどの細胞に共通して認められた。バンド染色によって、その起源は 11 番染色体の大部分と 1 番染色体の長腕部分との転座で生じたもので、その構造は、 $t(11pter \rightarrow 11q23::1q11 \rightarrow 1qter)$ であることがわかった。20 代目と 46 代目に再び染色体分析を行ったが、5 代目と同様に染色体数は 47 にモードを有し、すでに存在していたマーカー染色体も変わらず、認められた。なお、この細胞株の染色体分析で HSR (homogeneously staining regions) と DMS (double minutes) 所見はみられなかった。

B15. (Cancelled)

B16. ヒト T 細胞培養株の細胞遺伝学的研究：阿部達生・西田一弘・谷脇雅史・瀧野辰郎 (京都府医大・三内)、山根洋子・島田信男 (同臨検)、広瀬正雄・湊 啓輔・下山正徳 (国立がんセンター・内科). Cytogenetic Studies on 11 Established T-Cell Lines Derived from Human Leukemia: Tatsuo ABE, Kazuhiro NISHIDA, Masafumi TANIWAKI, Tatsuro TAKINO (Dept. Med., Kyoto Pref. Univ. Med.), Yoko YAMANE, Nobuo SHIMADA (Dept. Lab. Med., Kyoto Pref. Univ. Med., Kyoto), Masao HIROSE, Keisuke MINATO and Masanori SHIMOYAMA (Dept. Med., Natl. Cancer Cent., Tokyo)

ヒト白血病由来 T 細胞性永代樹立細胞株について、共通する染色体異常の存在を明らかにする目的で解析を試みたので報告する。株細胞は国立がんセンター内科で樹立あるいは保持されている継代培養中のもので、ALL 由来の 1) CCRF-CEM, 2) CCRF-HSB-2, 3) HPB-ALL, 4) JM, 5) KE-37, 6) MOLT-3, 7) p12/Ichikawa, 8) Peer, 9) Sommer と CLL 由来の 10) SKW-3, ならびに ALT 由来の 11) MT-1 の計 11 種である (以下、所見は番号で示す)。

G-, C- 染色や DAPI, アクチノマイシン D 等を用いる連続染色法などを用いて複雑な異常の解析を行った。染色体数 mode は diploid 域 7 株 (2, 3, 4, 5, 8, 10, 11) と tetraploid 域 4 株 (1, 6, 7, 9) であった。これら全株において相互転座を含む多彩な構造異常がみられた。これに関与した染色体を頻度の多い順に記載すると No. 2 (2, 3, 4, 5, 9, 11), No. 3 (1, 2, 7, 9, 10, 11), No. 6 (2, 6, 8, 9), No. 14 (3, 7, 9, 10, 11), No. 16 (2, 5, 6, 10, 11) のごとくであった。ことに (1) No. 2 の p23 (3, 4, 5, 9), No. 3 の p27 から q21 (1, 2, 7, 9, 10, 11), No. 6 の q21 から q25 (2, 6, 8, 9, 11) に切断点が多くみられたこと, (2) No. 14 における q13 から qter までの segment の関与した derivative な染色体 (7, 9, 10) さらに (3) No. 16 を含む dicentric 染色体 (2, 6, 11) がそれぞれの株に 1 個ないしそれ以上観察されたことは、これらに T-cell line に共通する異常の存在を示唆するものと考えられる。

B17. Cytogenetic Study of Malignant Diseases in Childhood (VI). Study for ph(+)
ALL: Ryuichi TSUKINO, Hiromitsu OMORI, Michio KOIKE (Dept. Pediat. Wakayama Med. Coll., Wakayama), Takashi KATO, Akira KAMIYA and Makoto SAKURAI (Dept. Pediat., Mie Univ., Tu)

Cytogenetic analysis were carried out for 3-year-old female with ph(+) ALL. She achieved complete remission after 4 weeks under the treatment of VCR, dexamethasone

and L-asparaginase and is good control for these 10 months.

Chromosome analysis were carried on bone marrow and peripheral blood with or without addition of mitogens (PHA and PWM) for three times, the first; at onset before treatment, 2nd; 3 months after remission and 3rd; 9 months.

A Philadelphia chromosome was found in the 1st sample (unstimulated blood) and 2nd sample (stimulated by PWM blood), but could not be found in the last sample. However, of bone marrow specimen obtained at the same time, 97% of metaphase cells had a Philadelphia chromosome. Various structural abnormalities such as dicentric, acentric fragment, ring, gap *etc.* were also found only in stimulated bloods.

B18. Chromosome Analysis of Tumorigenicity in Somatic Cell Hybrids between Malignant Mouse Cells and Human Bone Marrow Cells from Chronic Myeloid Leukemic Patients: M. C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Interspecific hybrid cells, resulted from fusion of bone marrow cells of chronic myeloid leukemic patients and malignant mouse LMTK-C1-1D or FM3A cells, analyzed for their tumor forming capacity in nude mice. Partial suppression of tumorigenicity was observed in several hybrid cells. Chromosome analysis of the hybrid cells before inoculation in nude mice and of the derived tumors revealed that human chromosomes 2, 8, 9, 11 and 13 were preferentially lost in any of the tumor cells in CML $\times$ 1D, and 7, 8, 9 and 20 in the tumors of CML $\times$ FM3A hybrids. These results suggest that no single human chromosome is responsible for the suppression of the tumorigenicity. The Ph¹ (22q-) chromosome in the leukemic cells was found in both tumorigenic and partially suppressed hybrid clones, indicating that Ph¹ chromosome had no influence on the tumorigenic behavior of the hybrid cells tested.

B19. Evaluation of the Micronucleus Test Using Human Leukemia Patients Treated with Anti-Leukemic Agents: Tatsuo ABE, Takuji ISEMURA (Dept. Med. Lab. Med., Kyoto Pref. Univ. Med., Kyoto) and Yasumoto KIKUCHI (Cent. Res. Div., Takeda Chem. Indust. Ltd., Osaka)

The relevance of the micronucleus test to human studies was investigated using bone marrow from leukemic patients treated with anti-leukemic agents. Micronuclei appear in the erythrocytes, especially polychromatic erythrocytes, of adult mice bone marrow after treatment with mutagenic compounds. However, it has not been determined whether micronuclei appear in erythrocytes of human bone marrow after treatment with mutagenic compounds. Bone marrow was aspirated from 10 patients with acute leukemia who had been treated with various chemotherapeutic agents in a single or combined regimens. Ten patients with diseases other than leukemia chosen as controls: 6 had not received any

drugs, whereas 4 had treatment with drugs other than anti-leukemic agents. The median incidence of micronucleated erythrocytes and erythroblasts respectively increased from control values of 0.04% and 0.72% to 0.29% and 25.3% in leukemic cases; and the frequency of micronucleated erythroblasts was inevitably higher than the control value in cases that showed a higher frequency of micronucleated erythrocytes, but the reverse was not true. These results indicate that almost the same changes of micronucleus formation that are observed in the mouse micronucleus test are produced in human bone marrow by anti-leukemic drugs-mutagenic compounds-, and if the micronuclei were scored restrictively in erythroblasts, the application of the micronucleus test to human bone marrow would be reasonable.

B20. Sister Chromatid Exchanges in Lymphocytes from Cigarette Smokers: Toshiaki WATANABE, Toshihiko KASUKAWA and Akira ENDO (Dept. Hyg. Prev. Med., Yamagata Univ. Sch. Med., Yamagata)

Recently cigarette smoking has been described in relation to possible increase of sister chromatid exchanges (SCEs) frequencies. However, the results are controversial as yet. So, the frequencies of SCEs and cell cycle kinetics were examined in cultured lymphocytes of 35 medical students and compared with between cigarette smokers (17) and nonsmokers (18). Also comparison was made on the susceptibility of lymphocytes to mitomycin C (MMC) (15 ng/ml) benzo(a)pyrene (BaP) (10^{-6} or 10^{-7} mol) between smokers and nonsmokers. For each individual 30 sets of metaphase were scored for SCEs.

The spontaneous SCEs frequencies in cigarette smokers were 10.3, ranging from 8.1 to 11.9, as compared with 9.6 (7.6–11.9) for nonsmokers, on average. This difference was marginally significant ($0.05 < p < 0.1$, by Mann-Whitney U test). However, SCEs were not correlated with the number of cigarettes smoked daily or the length of smoking habits. The SCE increases induced by MMC or BaP in lymphocytes were not different between smokers and nonsmokers. The extent of MMC- or BaP-induced delay of cell replication cycles was not different between two groups. This study suggests that cigarette smoking may affect DNA replication/repair and induce SCEs.

The SCE analysis may give valuable information on the effects of *in vivo* exposure to potential mutagens, if the sample size of populations to be examined is properly designed to assure certain level of statistical power of detection.

- B21. アルコール症者の培養リンパ球における染色体異常について：平井百樹（東大・理・人類）、原田勝二（筑波大・社会医学）、高木 敏・荒井正夫・海老原洋子・奥野府夫・重田洋介・河野裕明（国立療養所久里浜病院）. *Cytogenetic Studies of Japanese Alcoholics: Momoki HIRAI* (Dept. Anthropol., Univ. Tokyo, Tokyo), Shoji HARADA (Inst. Community Med., Univ. Tsukuba, Ibaraki), Satoshi TAKAGI, Masao ARAI, Yoko EBIHARA, Yorio OKUNO, Yosuke SHIGETA and Hiroaki KONO (Kurihama Natl. Hosp., Kurihama)**

人類のアルコールとのかかわりの歴史は長い。アルコールの代謝産物の一つであるアセトアルデヒドは細胞毒性が強く、クロスリンクとしての作用もある。このアセトアルデヒドを分解するアルデヒド脱水素酵素 (ALDH) のうちの低 K_m 値 ALDH 欠損型が、日本人などモンゴロイドで半数を占めることの生物学的意義は明らかでない。日本人のアルコール症者の ALDH 正常型と欠損型について、体内アセトアルデヒド濃度に生ずるであろう差がもたらす影響を知る研究の一環として、染色体レベルの調査を行った。

姉妹染色分体交換 (SCE) 頻度に関しては、ALDH 正常型 (20 名) と ALDH 欠損型 (2 名) においてともに上昇が見られたが、両者間に顕著な差は見いだされなかった (それぞれの平均値 12.78, 13.93)。SCE 誘発について、喫煙、他の常用薬などの影響を完全に除外することは不可能なので、今回の結果の解釈は慎重でなければならない。しかし、おそらく ALDH 正常型においても長期飲酒による酵素活性低下にともなう体内アセトアルデヒド濃度上昇が起こり、ALDH 欠損型と有意差のない SCE 誘発をもたらしたものと考えられる。

- B22. Induction of Sister Chromatid Exchanges in Human Lymphocytes by Steroids Used in Contraceptive Pills: Som-Arch WONGKHOMTHONG, Kanehisa MORI-MOTO and Akira KOIZUMI** (Dept. Public Health, Univ. Tokyo, Tokyo)

Human lymphocytes were treated with one of the eight steroid hormones used in contraceptive pills (Medroxy Progesterone Acetate, Norethinodrel, Norethindrone, Norethindrone Acetate, Ethynodiol Acetate/Ethynylestradiol, Norgestrel/Ethynylestradiol, Mestranol, and Ethynylestradiol).

The results show that these steroids have been categorized into three groups; first group (Mestranol and Norgestrel/E.E.) that gave a doubling of sister chromatid exchange (SCE) frequencies; second group (MPA, Ethynodiol Acetate/E.E., Norethinodrel, and Ethynylestradiol) that gave a slight increase in SCE frequencies; third group (Norethindrone Acetate and Norethindrone) that gave almost no increase in SCE frequencies. The data indicate that some steroids in contraceptive pills have the ability to induce SCEs in human lymphocytes. These results were discussed with previously reported data that the SCE frequencies in lymphocytes from oral-contraceptive pill users were higher than those in non oral-contraceptive pill users.

B23. Effects of Cycloheximide—a Protein-Synthesis Inhibitor—on the Base-Line and Mitomycin-Induced Sister Chromatid Exchange Frequencies in Human Lymphocytes: Kanehisa MORIMOTO, Mayumi SATO and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

Cycloheximide (CHX), a protein-synthesis inhibitor, has been reported to decrease the sister chromatid exchange (SCE) frequency in human fibroblasts and Chinese hamster cells. Experiments have been performed to examine if CHX could also decrease the frequencies of SCEs in cultured human lymphocytes. The results showed that, with increasing concentrations (2×10^{-8} M– 8×10^{-7} M) of CHX, cells exhibited a clearly dose-dependent delay in cell turnover times, but had constant base-line on mitomycin C (MMC)-induced frequencies of SCEs. Possible explanations for the discrepancy between the data reported on human fibroblasts and mammalian cells and those in the present lymphocyte study will be discussed.

B24. Effects of Cell Culture Conditions on the SCE Induction by DNA-Damaging Agents: Mayumi SATO, Kanehisa MORIMOTO and Akira KOIZUMI (Dept. Public Health, Fac. Med., Univ. Tokyo, Tokyo)

The base line SCE frequency were shown to increase with increasing culture times; Cells cultured in FBS-free medium consistently having a higher frequency of SCE than those cultured in 15%-FBS-added medium.

Experiments were also carried out to investigate the effects of incubation times between treatments with mitomycin C (MMC) or ethyl methane sulfonate (EMS) and the addition of 5-bromodeoxyuridine (BrdUrd). Cells were treated with 3×10^{-6} M MMC or 3×10^{-2} M EMS for 1 hr immediately before the start of cultures, and then incubated for different time periods (60–144 hr) with PHA. BrdUrd was added to culture medium for the last 42 hr of incubation.

Both MMC and EMS induced DNA damage that could lead to SCE formation. The data indicate that the induced DNA damage might be removed in time, but that the damage induced by EMS can be removed more quickly than that induced by MMC.

B25. Removal of Chemical-Induced Lesions that Lead to SCE Formation in Human Lymphocyte Cultures: Kumiko IJIMA (Dept. Matern. Child Health, Univ. Tokyo), Kanehisa MORIMOTO (Dept. Public Health, Univ. Tokyo, Tokyo), Makoto HIGURASHI (Dept. Public Health, Yamanashi Med. Sch., Yamanashi), Munehiro HIRAYAMA (Dept. Matern. Child Health, Univ. Tokyo) and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

The induction of sister chromatid exchanges (SCEs) has been regarded as a sensitive indicator of DNA damage. It is necessary for cells to pass through the S phase before

DNA damage in the cells can result in the formation of SCEs. If induced DNA damage is removed or repaired completely before cells enter S, then those cells show no increase in SCE frequency.

The human peripheral lymphocytes were cultured for 72 hr in the medium containing 5-bromodeoxyuridine. Cells were pulse treated with mitomycin-C (MMC), ethyl methane sulphonate (EMS), or 4-nitroquinoline-1-oxide (4NQO) along with a low level of tritiated thymidine at different cell cycle stages after the start of incubation.

The induction of SCEs were highest when cells were treated in early S phase by MMC (3×10^{-6} M) or EMS (2×10^{-3} M). The SCE frequencies reached about three-quarters of the highest values in cells treated at early second S phase. These data show that SCE forming the lesions might be similar in MMC and EMS treatment, thus suggesting only mono-adducts produce being major lesions that could lead to SCE formation.

B26. Proliferative Kinetics and Mitomycin C-Induced Chromosomal Damage in Fanconi's Anemia Lymphocytes: Kunihiko MIURA, Kanehisa MORIMOTO and Akira KOIZUMI (Dept. Public Health, Univ. Tokyo, Tokyo)

Lymphocytes from two sisters with Fanconi's anemia (FA) were studied for cell cycle kinetics, sister chromatid exchanges (SCEs), and chromosomal aberrations when they had undergone one, two, or three or more divisions in mitomycin C (MMC)-treated cultures. Lymphocytes from the parents and another sister of the probands and a healthy, unrelated adult were examined as normal controls. Analyses of cell cycle kinetics by the sister chromatid differential staining method revealed that the relative frequency of metaphase cells at their third or subsequent division was much smaller in untreated FA cultures than in normal cultures fixed at 96 hr after phytohemagglutinin stimulation. These data indicate that FA cells proliferate much more slowly than normal cells. MMC treatments of FA and normal cells led to a clearly dose-related delay in cell turnover times, the duration of delay being much longer in FA than in normal cells. FA cells had about 1.4 times higher frequencies of SCEs than normal cells in both MMC-treated and untreated cultures. FA cells also showed several times higher frequencies of chromosomal aberrations than normal cells, and the frequency of chromosomal aberrations decreased through subsequent mitoses by approximately 60% in both FA and normal cells.

B27. Chromosomal Sensitivity to 4NQO in Peripheral Lymphocytes of a Patient with r(10): Kunikazu KISHI, Tatsuro IKEUCHI, Kohtaro YAMAMOTO, Akira TONOMURA (Dept. Cytogenet., Tokyo Med. Dent. Univ.), Masako TOMITA, Noriyuki SAKURADA (Dept. Pediat., Tokyo Med. Dent. Univ.) and Yoshiaki SATOH (Dept. Dermatol., Tokyo Med. Dent. Univ., Tokyo)

The present patient showed some clinical features, such as growth retardation, mild mental retardation (EQ; 95), undescendent testes and a strawberry mark on abdomen.

Chromosome examination of the patient revealed a ring chromosome of No. 10. His mother explained that he had been sensitive against sunlight. Slight pigmentation due to sunburning and blistering is still remaining on arms and legs.

Because of his history of sensitivity against sunlight, peripheral blood lymphocytes of the patient were treated with $5 \mu\text{M}$ 4-nitroquinoline-1-oxide (4NQO) for 24 hr in order to examine the chromosomal sensitivity. Significantly high frequency of chromatid aberrations (0.893 per cell) was observed in patient's lymphocytes compared with control (0.080 per cell) ($p < 0.001$). The analysis of the cell cycle stage sensitivity revealed that the chromosomes of his lymphocytes were hypersensitive at S and G_2 phases. Cultured fibroblastic cells obtained from the skin, however, did not show high chromosomal sensitivity to $0.5 \mu\text{M}$ 4NQO.

Quantitative data of the patient for minimal erythema dose (MED) obtained at 24 hr post UV irradiation revealed normal responses at the age of 2.5 years. At present, the difference of chromosomal sensitivities between lymphocytes and skin fibroblasts to 4NQO is not known.

B28. The Effects of Scavengers to γ -Ray Induced Chromosome Aberrations in the Lymphocytes with 21 Trisomy. I. Cysteamine: Fumiko SAITO and Akira TONOMURA (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

The frequency of exchange-type chromosome aberrations induced by γ -ray irradiation in trisomic cells is significantly high compared with normal cells. In normal cells, protective effects against the production of this type of aberrations have been found in some drugs (scavengers) which scavenge some free radicals produced by indirect action of γ -ray irradiation. To examine the effects of these scavengers on trisomic cells, cysteamine treatment was carried out in lymphocytes obtained from patients with Down's syndrome.

The lymphocytes from Down's syndrome and normal control were treated with cysteamine at various concentrations (0, 0.5, 1, 2.5, 5, 10 and 50 mM) and the cells were irradiated by γ -ray (300R, 112R/min), washed twice and then incubated for 48 hr at 37°C in NCTC-109 supplemented with 20% fetal calf serum. The chromosome preparations were made according to the standard procedure. The frequencies of discs.+ rings were 0.51, 0.41, 0.37, 0.25, 0.20, 0.12, and 0.11 per cell in the control and 0.67, 0.62, 0.49, 0.35, 0.26, 0.20 and 0.15 per cell in the trisomy at various concentrations of cysteamine. These results showed that the reduction rate of the aberrations in the trisomic cells were comparable to that in the normal cells. The frequency of the terminal deletions was decreased with the concentration of cysteamine in normal and trisomic cells. When 21 trisomic cells were exposed to 100, 200 and 500R at 5 mM cysteamine, the frequencies of exchange-type aberrations were reduced about one half. These results suggest that cysteamine has protective effects against irradiation in the trisomic cells as well as in the normal cells on the production of chromosome aberrations.

B29. A Case of 46,XX/47,XXXq-: Masaru KOIKE, Shun SATO, Noriyuki HIRAI
(Dept. Clin. Lab., Jikei Univ.), **Sachio IKAWA** (Dept. Clin. Med., Jikei Univ.),
Naoki KAMIYA and Masaharu IWATA (Dept. Gynec., Jikei Univ., Tokyo)

A 21-year-old female with secondary amenorrhea was found to have a karyotype 46,XX/47,XXXq-. On physical examinations she was 153 cm tall and weighed 50 kg. Mental retardation, gross malformation and stigmata of Turner syndrome were not observed.

Urinary excretion of 17-OHCS and 17-KS were 1.4 mg/day, 4.7 mg/day respectively. Levels of PRL, GH, TSH, E₂, testosterone and progesterone in her serum were normal range. In LH-RH tests LH and FSH of the baseline were 100 mIU/ml and 130 mIU/ml respectively, and these levels increased higher than the normal response.

Chromosomal analysis was performed by culture methods using peripheral blood. The karyotype was evaluated using Q-banding and R-banding. The karyotype revealed two types of cells by Q-banding. One type had 46,XX and another had 47 chromosomes including an unusual shape and small abnormal chromosome. This abnormal chromosome was similar to 4p, 5p, 10p, Xp, and 16q. However, with R-banding, the abnormal chromosome confirmed to be short arm of late replicating X. A frequency of 47,XXXq- cell was 3.6% among 300 cells. Thus, the patient was a mosaic of 46,XX/47,XXXq-.

B30. Partial Trisomy 8q Resulting from Maternal Balanced Translocation t(8;18)(q22;q22): Yutaka SATO, Susumu SUNAGA, Akiko SAWA and Kazuo OKU-YAMA
(Dept. Pediat., Showa Univ., Tokyo)

Recent years partial trisomy 8q has proven to be a clinically recognizable syndrome owing to many previous reports. We report clinical observations and cytogenetic studies of an inherited form of partial trisomy 8q.

This girl, born 2,335 g, was the first child of nonconsanguineous parents. Delivery took place at 41 weeks' gestation. The following clinical features were found: hypotonia, poor sucking, failure to thrive, cleft lip, cleft palate, hyperetorism, short nose and broad base, low-set ears, short neck, congenital heart disease (acyanotic tetralogy of Fallot), hydro-nephrosis, overlapping of the fourth and fifth toes. Dermatoglyphic analysis showed bilaterally displaced axial palmar triradii.

Chromosome examinations from blood lymphocyte cultures were performed on this patient and several family members. The karyotype of this patient exhibited 18q⁺ chromosome. The father's karyotype was normal but the mother's and maternal uncle karyotypes revealed a balanced translocation between chromosomes 8 and 18. G-banding and R-banding identified the maternal formula as 46,XX,t(8;18)(q22;q22) and the uncle's formula as 46,XY,t(8;18)(q22;q22). Thus the derived maternal No. 18 chromosome had segregated in the proposita, and she was trisomic for region 8q22→qter. Because her karyotype was decided 46,XX,der(18),t(8;18)(q22;q22)mat.

B31. A Case of Partial 7q Trisomy: Tamiko SHINOHARA (Dept. Hum. Cytogenet., Japan Red Cross Med. Cent.), **Yoshiharu SONE and Hiroshi AKAMATSU** (Dept. Neonatal, Japan Red Cross Med. Cent., Tokyo)

The propoita was born on May 18, 1981, after 38-week-6-day-gestational period, as the first pregnancy of a 26-year-old mother and a 28-year-old father. Both were in good health and not consanguineous. The pregnancy was complicated with cepharopelvic disproportion. The baby was delivered through the vagina by means of a vacuum extraction. Apgar 5. Birth weight was 2,132 g, length 46 cm. Head and chest circumference were 35.5 cm and 27.5 cm, respectively. At birth, the malformations were observed: a prominent occiput and frontal bossing, thick hair, narrow and small palpebral fissures and entropion cilia, flat nasal bridge, low-set ears with large helixs, short neck, clinodactyly of the fingers and single transverse palmar crease on right hand. She was slow to feed initially, growth and development have been delayed. At the age of 17 months, her weight was 8,500 g, length 75 cm. She could not hold her head and was also unable to sit. She appeared to hear but had no intelligible speech. Scoliosis was present. CT-scan and EEG revealed abnormal findings.

Chromosome preparations were made from peripheral blood lymphocytes. G-band, C-band and silver stain were performed. The karyotype of the patient was identified as 46,XX,-14,+der(14)t(7;14)(q31;p13)_{mat},15s+, and her mother's karyotype was 46,XX,t(7;14)(q31;p13),15s+. So the patient was designated as partial trisomy 7q31→7qter. On the other hand, the karyotypes of the sister and brother of her mother were 45,XX,-13,-14,+t(13q14q),15s+ and 45,XY,-13,-14,+t(13q14q), respectively. The maternal grandmother of the patient had these two types of translocation, that is, 45,XX,-13,-14,t(7;14)(q31;p13),+t(13q14q). The maternal grandfather had 46,XY,15s+. The patient's mother, uncle and aunt, grandfather and grandmother were phenotypically normal. The mother's second pregnancy has been prenatally diagnosed as 46,XY,t(7;14)(q31;p13) by chromosome analysis of amniotic cells.

B32. 次端着糸型染色体部分トリソミーの分離様式; +(14q-), +(15q-) 各 1 例を経験して: 谷川洋子 (兵庫県塚口病院・検査), 早川 泰 (同・小児), 藤田弘子 (大阪市大・生活科学). Study of Disjunction for Produced to the Partial Trisomy of Acrocentric Chromosome, Including of Our Patient with 14q Proximal Trisomy and One with 15q Proximal Trisomy: Yoko TANIGAWA (Hyogo Pref. Tsukaguchi Lab.), **Yasushi HAYAKAWA** (Dept. Pediat., Hyogo Pref. Tsukaguchi Hosp., Hyogo) and **Hiroko FUJITA** (Osaka City Univ., Osaka)

症例 1. 14q 近位部過剰症の典型的症状をもつ 2 歳女児. 47,XX,der(14),t(2;14)(p25;q24)_{mat}. 母は自然流産 2 回と満期死産児の妊娠歴がある. 症例 2. 知能障害と小顎, 仙骨洞など 2~3 の外表奇

型をもつ 2½ 歳女児, 47,XX,der(15),t(2;15)(q37;q15)mat. 兄 2 人でうち 1 人は母と同じ相互転座である。

細胞遺伝学的文献考察: 相互転座をもつ親から生まれる三次端部着糸型部分トリソミーは、減数分裂パキテン期でいかなる分離様式が実際に起こるか、DG 群について文献的に調査した。対象は、上記自験例を含む 65 の近位部トリソミーと 29 の遠位部トリソミー発端者である。結果は次のごとくである。

1) 近位部トリソミーは、3:1 分離 60 (うち 53 は Tertiary トリソミー) と隣接分離 2 型, 5 があり、隣接分離 1 型の例は見当たらない。2) 遠位部トリソミーは、隣接分離 1 型 25, 3:1 分離 4 があり、隣接分離 2 型の報告例なし。3) 隣接分離 2 型は、まれな分離様式であるが同胞 2 人と 3 人連続出産が 5 家系中各 1 家族にみられ、再発率の高い分離様式と思われる。遺伝相談上注意を要する。

B33. Cytogenetic Study of Familial Prader-Willi Syndrome: Tomoko HASEGAWA, Kiyomi YAMADA (Div. Genet., Natl. Med. Cent., Tokyo), Michiko HARA, Michiko ANDO, Makiko OHSAWA and Yukio FUKUYAMA (Dept. Pediat., Tokyo Women's Med. Coll., Tokyo)

We present cytogenetic findings in two cousins with Prader-Willi syndrome, their healthy parents and grandparents.

Clinical findings and family history. Proband was a 10-year-old obese boy, who had severe mental retardation, muscle hypotonia in early infancy, white skin color, myoclonic seizure, almond-shaped eyes, upsweep of frontal scalp, decayed teeth, small hands and feet, hypertrichosis, cryptorchidism and small penis and scrotum. He had recurrent infection. At the age of 3 he was diagnosed as Prader-Willi syndrome. Before his birth the mother had 2 pregnancies. An abortion and a 4-month-lived girl who died of pneumonia. The appearance of the girl was very similar to his. The mother had healthy sister and brother. Other 6 sibs in early infancy. The daughter of the brother was 4 years old and severely retarded. The girl had hypotonia, white skin, almond-shaped eyes, hypotelorism, small hands and feet, hypertrichosis and genital hypoplasia, but no seizure. She was also diagnosed as Prader-Willi syndrome.

Cytogenetic findings. The proband had 46 chromosome. The paracentromeric area of a 15q had some difference from the homologous chromosome. By the sequential staining of DA/DAPI and AMD/DAPI an intense DA/DAPI band on a 15q and the homolog had negative with DA/DAPI. The finding was similar to the cousin's. The mother of the proband had two intense DA/DAPI bands, but on a no. 15 and no. 14. The brother of the mother and the grandmother of the proband had also bright DA/DAPI bands on the different chromosomes. From these results we estimated reciprocal translocation of no. 14 and no. 15 in the mother, uncle and the grandmother of the proband. The father of the proband and the mother of his cousin had normal karyotypes. The break points were assumed to be 14q11.2 and 15q13 by the high resolution-G-banding procedures. The

affected cousins had partial trisomy 14pter→14q11.2 and partial monosomy 15pter→15q13.

B34. A Male Case with Pure *de novo* 9p Trisomy, High Activity of Galactose-1-Phosphate Uridyltransferase: Atsushi IESHIMA and Kunio YOSHINO (Div. Child Neurol., Tottori Univ., Yonago)

A 2-year-old boy with trisomy 9p was presented. High activity of galactose-1-phosphate uridyltransferase (GALT) in red blood cells was demonstrated.

The clinical features of the proband included short stature (-2.5 SD), psychomotor retardation (DQ=53), brachycephaly without microcephaly (-0.8 SD), big nose, high nasal bridge, low-set malformed large ears, shark-shaped mouth, short neck, tapering brachydactyly, clinodactyly of the fifth fingers, bilateral simian crease, and talipes planovalgus. Dermatoglyphics revealed characteristic abnormalities: axial triradii in the distal position (t'', t''), bilateral thenar pattern, absence of the b, c-line (Lt), second and third interdigital pattern (Rt), decreased total finger ridge count (70), tibial arch on the both hallucal areas.

Radiological study showed delayed bone age, hypoplastic distal phalanges of the fingers and toes, and brachymesophalangy of the fifth fingers. All these findings were compatible with those of 9p trisomy.

The computed tomography of the head demonstrated disproportional dilatation of the lateral ventricles, which was colpocephalic.

Cytogenetic studies used with lymphocyte and fibroblast culture revealed the karyotype of 47,XY,+del(9)(pter→q12;) by the C-band and high-resolution G-band techniques. The parental karyotypes were normal.

The GALT activities in red blood cells were assayed by Beutler's method. The GALT activities of the proband were 50.6, while those of normal controls (7 cases) were 26.7 ± 7.2 . This result supports a gene dosage effect for GALT in 9p trisomy.

B35. Regional Mapping of GLO and HLA on Human Chromosome 6 Using Blood Obtained from Two Cases of 6p Trisomy and One Case of 6p Monosomy: Akira HATA, Yoshimitsu FUKUSHIMA, Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama) and Kazunobu OUCHI (Dept. Pediat., Natl. Okayama Hosp., Okayama)

The gene loci for HLA and GLO (glyoxalase-I) have previously been assigned and confirmed to the short arm of human chromosome 6. Regional mapping studies have shown that HLA is in the 6p2105 to 6p22 region whereas GLO is in the 6p2100 to 6p22 (HGM 6,1981). We investigated the expression of HLA and GLO using blood obtained from 2 cases of 6p trisomy (46,XX, inv dup(6)(p25→p21.3) and 46,XX,t(6;15)(p21.5;p11)) and one case of 6p monosomy (46,XX,del(6)(p23)). HLA typing showed no excessive or defi-

cient gene, allowing the assignment of HLA to the 6p2105 to 6p21.3. For GLO assay, 200 μ l 10 mM methylglyoxal as substrate, 200 μ l 30 mM reduced glutathion, 200 μ l 100 mM $MgCl_2$, 1 ml 100 mM PBS pH 6.8, 350 μ l D.W., 50 μ l hemolysate as enzyme source were mixed and production rate of thioester (Abs 240 nm) was measured. The activity showed no significant difference in comparison with normal controls, which indicates the assignment of GLO to the 6p2100 to 6p21.3 if gene does effect exists.

B36. Regional Mapping of Human Chromosome 13. Assignment of the Gene for Esterase D to 13q13→q22 : Hidetsune OISHI, Itsuro NISHIGAKI (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony), Masahide FUTAMURA, Tsutomu YAMANAKA and Mitsuo KUROYANAGI (Cent. Hosp., Aichi Pref. Colony, Kasugai)

The gene locus for esterase D has been assigned to chromosome 13 by somatic cell hybridization analysis. However, an inconsistent result for regional mapping on this chromosome was obtained from 7 data accumulated, including our previous finding in a partial deletion of chromosome 13 (Human Gene Mapping 5, 1979). In the present study, we report an additional case with structural abnormality of chromosome 13, whose chromosomes were investigated by the trypsin and fluorescent banding techniques. Isozyme patterns of esterase D in the red cell of the patient and his parents were also examined.

The patient was born Jan. 28, 1982, after 40 weeks gestation, as the first child of a 29-year-old mother and a 28-year-old father. The parents were not consanguineous. At birth, the boy was found to have multiple abnormalities, such as found in Patau's syndrome. The karyotype of the patient was 46,XY,der(4),t(4;13)(q35;q13)mat. His father had normal chromosomes.

The electrophoretic pattern of the patient was basically ESD 2-1, but the proportion of enzyme activity in the bands suggested ESD 2-1-1, showing an apparent gene dosage relationship of this enzyme in partial trisomy 13. The isozyme patterns of his mother and father were ESD 2-1 and ESD 2, respectively.

Since the *ESD* locus in the previous study is excluded from the region 13q22→qter, the data suggest that *ESD* activity is controlled by a gene located on chromosome 13 within region q13→q22.

B37. Chromosome Abnormalities Detected in Couples with Repeated Spontaneous Abortions: Kaoru SUZUMORI, Takiko KOISHI, Takako HISAOKA, Katsuhide IMAIZUMI and Yoshiaki YAGAMI (Dept. Obst. Gynec., Nagoya City Univ., Nagoya)

A number of investigators reported that more than 50% of spontaneously aborted fetuses were cytogenetically abnormal. On the basis of these results, the second step is to identify

possible cytogenetic defects in either parent that may predispose to recurrent abortions. The group under study comprised 73 couples who had experienced three or more spontaneous abortions. In each case, both members of the couples were examined. For chromosome analysis, R-, Q-banding and in some case G-banding techniques were used. Of these, 3 demonstrated balanced chromosomal translocation in anyone of the couples (3/73, 4.1%). The abnormal karyotypes observed were 46,XY,-4,-4,-6,-10,+t(4p6p), +t(4p10q),+t(4q6q),+t(4q10p),46,XY,t(8;13)(q23;q21), and 46,XX,-6,-10,+t(6p10p), +t(6q10q). In 1979, Daniel has attempted to distinguish couples with recurrent spontaneous abortions without full-term abnormal offspring from those with unbalanced neonates by measuring the interchange segment sizes. Our results also support this view that the interchange segments were larger in the spontaneous abortion reciprocal translocation.

B38. ヒト胎児培養線維芽細胞の X 染色体後期複製パターン. 生後組織との比較: 塚原 正人・梶井 正 (山口大・小児). X Chromosomal Late Replicating Patterns of Cultured Fetal Fibroblasts. Comparison with Adult Fibroblasts: Masato TSUKAHARA, Tadashi KAJII (Dept. Pediat., Yamaguchi Univ. Sch. Med., Ube)

1) 人工流産胎児 (46,XX) の皮膚由来培養線維芽細胞, 2) 成人女性リンパ球細胞, 3) 同皮膚由来培養線維芽細胞の X 染色体後期複製パターンの差異について検討した. G_2 の長さ (分裂像のうち 50% が BrdU を取り込む時間) は, 1) 3.5 時間, 2) 2.5 時間, 3) 3.5 時間だった. 培養細胞 S 期後期に BrdU (最終濃度 25 $\mu\text{g/ml}$) を 1 時間作用させた後, 染色体標本を作成, Hoechst-Giemsa 法で染色, 写真撮影後 X 染色体各 50 個について分析した. 後期複製 X 染色体の比較では, 各領域の BrdU 取り込みの有無により, 1) 17 種, 2) 18 種, 3) 14 種のパターンが区別できた. 後期複製 X 染色体各領域の比較では早期複製するのは, 1) Xp11, Xq22-Xq24, 2) Xp22, Xp11, Xq11, Xq13, Xq26, 3) Xp11, Xq13, Xq22-Xq24 だった. リンパ球で Xp22, Xq26, 成人皮膚・胎児皮膚由来線維芽細胞で Xq22-Xq24 が早期複製するのが特徴だった. 後期複製するのは, 1) Xp21, Xq21, Xq25, Xq27, Xq28, 2) Xp21, Xq21, Xq22, Xq23, Xq24, Xq25, Xq27, Xq28, 3) Xp21, Xq21, Xq25, Xq27, Xq28 だった. リンパ球で Xq22-Xq24, 成人皮膚・胎児皮膚由来線維芽細胞で Xq26 が後期複製するのが特徴だった. 前期複製 X 染色体各領域の比較では, Xp21, Xq21, Xq25, Xq27 は三者ともに後期複製し, リンパ球で Xq23 が後期複製するのが差だった.

以上の結果から, 胎児皮膚由来培養線維芽細胞, 成人リンパ球細胞, 成人皮膚由来培養線維芽細胞の三者間で, X 染色体後期複製パターンに差があることを証明した.

B39. Allocyclic Early Replicating X Chromosome in Mice. Genetic Inactivity and Shift into a Late Replicator in Early Embryogenesis: Osamu SUGAWARA, Nobuo TAKAGI and Motomichi SASAKI (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

The allocyclic X chromosome in early female mouse embryos undergoes DNA replication either late or early in the S phase. Studies reported so far indicated that the early replicating X chromosome is restricted to the trophectoderm and primitive endoderm cell

lineages in which the allocyclic X is almost exclusively paternal in origin. They have provided, however, no compelling evidence for the genetic inactivity of the early replicating X chromosome and the shift from the early to late replication or *vice versa*. The present study employing a combination of ^3H -TdR autoradiography and BUdR labeling-acridine orange fluorescence staining in day-6 female mouse embryos furnished definitive evidence that the early replicating X chromosome can directly change into a late replicator. The activity state of the early replicating X chromosome was explored by the electrophoretic analysis of an X-linked enzyme phosphoglycerate kinase (PGK-1) in tissues isolated from 6.0- and 8.5-day *Pgk-I^a/Pgk-I^b* embryos. Only the maternally derived *Pgk-I* allele was expressed in the proximal endoderm and extra-embryonic ectoderm of 6.0-day embryos and the chorion of 8.5-day conceptuses. Thus, the early replicating, paternally derived X chromosome found in about 70–80% of cells constituting these tissues seems to be transcriptionally repressed as the late replicating one.

B40. The Late-Replicating X Chromosome in Digynous Mouse Triploid Embryos:

Sumiyo ENDO, Nobuo TAKAGI and Motomichi SASAKI (Chromosome Res.
Unit, Fac. Sci., Hokkaido Univ., Sapporo)

Using BrdU-labeling and acridine orange staining, the behavior of X chromosome replication was studied in 28 XXX and 19 XXY digynous mouse triploids. In some of these the paternal and maternal X chromosome could be cytologically distinguished. Such embryos were obtained by mating chromosomally normal females with males carrying Cattanach's X chromosome which contains an autosomal insertion that substantially increases the length of this chromosome. In the XXX triploids there were two distinct cell lines, one with two late-replicating X chromosomes, and the other with only one late-replicating X. The XXY triploids were also composed of two cell populations, one with a single late-replicating X and the other with no late-replicating X chromosome. Assuming that the late-replicating X is genetically inactive, in both XXX and XXY triploids, cells from the embryonic region tended to have only one active X chromosome, whereas those from the extra-embryonic membranes tended to have two active X chromosomes. The single active X chromosome was either paternal or maternal in origin, but two active X chromosomes were overwhelmingly maternal in origin, suggesting paternal X-inactivation in extra-embryonic tissues.

B41. Age and Hyperdiploidy in Human Lymphocytes: Jun-ichi MURAMOTO and

Kisaburo AKASAKA (Fukushima Biomed. Inst. Environ. Neoplas. Dis., Ohkumamachi, Fukushima)

We studied the proportion of hyperdiploid cells (≥ 47) which is expressed as a percentage of them per total count of each person in 34,678 cells from 354 normal females and 18,952

cells from 192 normal males varying age from 15 to 84 by the method of blood culture (48–50 hr). The data in both sexes found to increase with increasing age, namely they fit to a linear regression with age (XX, $y=0.01x+0.22$; XY, $y=0.01x+0.04$). The correlation coefficient (r) is 0.18 ($p=0.025$) for males and 0.23 ($p=0.01$) for females. Females show a high rate of increase of hyperdiploidy compared with that in males.

When the mean values are evaluated by finding of the mean of the sum of every age groups of 5 years, the data can give a better fit to a linear regression under the age groups of 65–69 years ($r=0.88$, $p=0.001$ for females; $r=0.76$, $p=0.01$ for males), while over 70 years of age, the trend in the data of both sexes rather decreases progressively with an increase in age. The reason of this interesting finding is still unknown and further studies must be done.

To know the types of chromosomes involved in the formation of the hyperdiploidy, an increase attempt has been made to ascertain the additional chromosomes belonging to which one of the seven groups based on the Denver classification. The additional chromosomes seem to belong mainly to the C-group in females (60.8%), while in males to the C-group (41.3%) and the groups other than the C and G groups (43.3%).

B42. The "Loss" of Kinetochores from Chromosomes of Aged Individuals: Yasuo NAKAGOME (Natl. Inst. Genet., Mishima) and Tatsuo ABE (Kyoto Pref. Univ., Kyoto)

It is widely known that both aneuploid cells in mitoses and non-disjunction in meiosis increase with advancing age. Cause(s) of these phenomena have not been known. In the present study, the Cd-band technique was applied on mitotic spreads obtained from aged individuals. It has been established that a positive Cd reaction represents the presence of an active kinetochore and a negative reaction either its inactivation or loss (Nakagome *et al.*, 1976, 1980; Daniel, 1979). Blood samples were obtained from 14 females (66 to 87y; $av.=78y4m$) and 11 controls ($av.=8y4m$). To avoid effects of possible technical variables, only cells in which all 6 members of the A-group showed Cd spots were included in the study. The remaining chromosomes were scored except for those with overlap.

In aged females, 56 chromosomes were Cd negative out of 6,476 scored (control: 11/3,057). The difference was highly significant ($p<0.01$). Similar (but slightly less) trend was observed in 2 aged males. Six of the 14 aged females were examined by either the C or the distamycin-DAPI technique in addition to the Cd. Thirteen out of 2,080 chromosomes showed separation of their kinetochores (control: 7/5,080). C-group chromosomes were most frequently involved in either types of abnormalities followed by the D and B groups. It was concluded that the kinetochore tended to lose Cd-positive material in aged individuals.

B43. 放射線の oocyte killing 効果を応用した卵子の生産勾配と染色体不分離の関係の検討： 立野裕幸・美甘和哉 (旭川医大・生物). A Reinvestigation on the Correlation between "Oocyte Production Line" and Meiotic Nondisjunction: H. TATEN0 and K. MIKAMO (Dept. Biol., Asahikawa Med. Coll., Asahikawa)

チャイニーズハムスターの生後0日の卵巣ではすべての卵子が放射線抵抗性の厚糸期にあり、放射線の oocyte killing 効果は全く認められない。一方、生後4日では多数の卵子は放射線高感受性の複糸期・網糸期に達し、照射後は少数の厚糸期卵子が選択的に残される。被照射個体は残存卵子数に応じて生殖可能月齢を短縮するが、性成熟開始月齢の遅延も、残存卵子への放射線晩発効果も、全く認められない。この放射線の選択的 oocyte killing 効果を応用して、Henderson と Edwards (1968) の卵子生産勾配と染色体不分離の関係を再検討した。

両照射群 (100 rad) が 4~5 カ月の最適生殖月齢に達したとき、受精卵の雌性前核の染色体分析を行い、卵子の成熟分裂期染色体不分離頻度を比較した。対照群、0 日照射群、4 日照射群の異数体出現頻度はそれぞれ 2/203 (1.0%), 1/144 (0.7%), 0/121 (0%) であった。

この結果は、遅れて成熟期に入った卵子でも母体の若年期に排卵された場合は、染色体不分離を起こさないことを証明するもので、卵子の生産順序が卵子染色体の分離行動を左右するものではないことを明快に説明するものである。

B44. Fragile Site 3p14—Cytogenetic and Clinical Studies: Mariko UEHARA and Mitsuhiro KIDA (Dept. Pediat., Teikyo Univ., Tokyo)

Chromosome analyses were carried out on 35 children with multiple minor anomalies, by means of medium 199 (poor folic acid) using peripheral blood cells, since Feb. 1980 to Oct. 1982.

The frequencies of fragile site 3p14 were high in three children A, B and C. In repeated studies the mean frequencies were 0.06/cell in A, 0.08/cell in B, 0.14/cell in C, and that of their mother were 0.10/cell, 0.05/cell and 0.03/cell. (The mean frequency was 0.01/cell in total of 35 children.)

Clinical findings were prominent forehead, epicanthal folds, narrow palpebral fissures, hyperterolism, strabismus, broad flat nasal ridge, saddle nose, ptosis of the upper lids, hypotonicity, delayed growth and psychomotor development in three children A, B and C. Simian crease, limit extension, epilepsy in two children. (These clinical features are seen in that of deletion 3p14 cases already reported.) Their mother were clinically normal.

The frequency of fragile site 3p14 was high by poor folic acid and low dose of colsemid in G₂ phase *in vitro*. These results suggest that expression of the fragile site 3p14 is influenced by DNA syntheses especially thymidine and coiling of chromatid or any other process in G₂ phase.

- B45. Staining Affinity of Formazan-Like Compounds to Human Chromosomes: Kou-ichi MAMBA, Kazuyuki TANIGUCHI, Mutsuo KITAHAMA** (Dept. Legal Med., St. Marianna Univ. Sch. Med., Kawasaki) **and Akira UCHIUMI** (Natl. Chem. Lab. Indust., Tsukuba)

Seven kinds of chelate reagents available in the market, that is, calcein, methyl calcein, methyl calcein blue, umbelliferone, NAM(*n*-(9-acridinyl) maleimide), NBD-F(4-fluoro-7-nitro-benzofurazan) and Morin, and five kinds of azo compounds newly synthesized, that is, BOAP(1-(2-benzoxothiazolylazo)phenanthrol), TAP(1-(2-benzothiazolylazo) phenanthrol), QAP(1-(2-quinolylazo)-9-phenanthrol), CAP(1-(2-carboxylazo)-9-phenanthrol and BTPCF(1-(2-benzthiazolyl)-3-(2-sulfophenyl)-5-(3-carboxylphenyl) formazan were examined by fluorescent light microscopy about their staining affinities as the fluorescent dyes to human chromosome samples. All the reagents and the compounds examined in the present study showed the good staining affinity to the chromosome samples, though R-band was recognized only by the double staining of umbelliferone and methyl green.

- B46. ふたごの近親におけるふたごの有無: 山田一朗・浅香昭雄** (東大・医・精神衛生).
Twins in the Relatives of Index Twins: Kazuaki YAMADA and Akio ASAKA
 (Dept. Mental Health, Univ. Tokyo, Tokyo)

昭和 23~56 年までに東大附属中に志願または入学したふたごのうち、卵性の明らかなもの 967 組を対象とした。資料は、志願時の志願票と、それをもとにして主に母親に面接したときの記録によった。近親にふたごのみられたものは、MZ 729 組中 289 組 (39.6%), DZ 238 組中 82 組 (34.5%) であった。そのうち、近親に、発端のふたごを含めて 3 組以上のふたごが多発している家系は MZ では 289 組 70 家系 (24.2%), DZ では 82 組中 22 家系 (26.8%) であった。

発端ふたごの同胞におけるふたごの再出現率を計算したところ、DZ の同胞に DZ が再現する率は出産 1,000 対 7.4 であった。この値は、日本人の一般人口中における DZ の出産率の 3.2 倍にあたる。また、異性ふたごの同胞における DZ の再現率が一般人口中より約 4 倍高いという Weinberg, Bulmer の報告とも一致する所見である。MZ において、母方近親にふたごのみられる家系 (124) のほうが、父方近親にみられる家系 (78) よりも多い。このことから、多胚化に関与する遺伝要因がもっぱら母によって表現されるという可能性も否定できない。また MZ, DZ の家系双方にほぼ同じ高率で近親にふたごが存在する家系がみられたことは、浸透率の低い優性遺伝子の存在を仮定することで、よく説明することができる。(本研究は、東京大学創立百年記念学内共同研究助成金によった。)

- B47. Dermatoglyphic Study of Female Patients with Diabetes Mellitus and Their Children: Ekuko NAKADA, Tatsuko FURUYA, Makiko OSAWA, Kiyoko YAMAGUCHI, Yukio FUKUYAMA** (Dept. Pediat., Tokyo Women's Med. Coll.) **and Yasue OMORI** (Diabetes Mellitus Cent., Dept. Intern. Med., Tokyo Women's Med. Coll., Tokyo)

It is generally known that the incidence of congenital malformation is high in the infant of diabetic mother. However, the pathogenesis and cause of it are unknown. With the

aim of contributing to the problem, dermatoglyphic analysis was undertaken in 69 female patients with diabetes mellitus (DM) (23 cases of type I and 46 of type II DM) and their 64 children (32 boys and 32 girls) in comparison to those of 110 healthy controls (60 boys and 50 girls) with no malformation. The subjects were selected from 93 female diabetic patients who gave children under the treatment at the DM Center of our hospital during the past 17 years.

Method. The ink stamping method was used for recording dermatoglyphs. The ink for the fingerprints was spread on a glass plate by an ink roller and then the palm and finger were printed.

Results. 1) Dermatoglyphic findings in diabetic mothers were different from the control in the following points: (1) The percentage of patients whose palmar alignment tended to be vertical and (2) that with absent interdigital pattern was significantly higher than the control. 2) The type II patients showed both a vertical main A line in the palmar alignment and absence of interdigital pattern more frequently in comparison to the type I patients. 3) As compared with the controls, the children from diabetic mothers showed a similar tendency in dermatoglyphic figures with their mothers, and in addition there were less percentage of children showing the L type thenar pattern. 4) In a few dermatoglyphic features, there were significant differences between the diabetic mother and their children. A further study should be warranted in more detail and in a larger extent.

B48. 化粧品障害の遺伝的背景について：大倉興司（東医歯大・難研・人類遺伝）.

Genetic Background for Skin Trouble Due to Exposure to Cosmetics: Koji OHKURA (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

化粧品による皮膚障害は一般消費者の 30% 程度にみられるとされ、家族的発生も指摘されているが、その遺伝的背景についての調査はない。本調査では、保健婦を主とした看護職に対する遺伝に関する研修会において、化粧品障害に関して、家系資料を含むアンケート調査を行った結果から、遺伝性を分析した。なお、家系調査では、調査対象は化粧品使用経験のある女性血族に限った。

回答者 818 名中、356 名 (43.5%) になんらかの障害が認められた。しかし、調査対象のうち無障害の者が一部回答していないので、この頻度はやや高いと考える。有障害者の家系中に有障害者を認めたのは 61.3% で、無障害者の家系では 24.2% であった。

有障害者 346 家系でその娘 64 名中 28 名 (43.8%) に障害がみられ、無障害者の娘では 109 名中の 11.0% であった。障害の原因となった化粧品は化粧水 (42.4%)、クリーム (34.8%)、乳液 (33.2%) 等で、症状ではかゆみ (67.4%)、ぶつぶつ (湿疹) (58.4%)、発赤 (49.2%) 等であった。

有障害者の娘 64 名中 28 名が障害を示し、分離比は 0.4375 で、優性遺伝の可能性を示唆した ($\chi^2 = 1.00$)。さらに全家系図中から母親で障害のあった 101 家系につき分離比を求めたところ、114 : 101, 0.5302 ($\chi^2 = 0.7860$) となった。しかし、父親に関する情報がないので、父親の 30% がヘテロ接合と仮定して期待値を求め、この観察値を検討したが、 $\chi^2 = 1.7623$ で、優性遺伝という仮説を否定す

ることができないことが明らかとなった。

なお、有障害者の両親の近親婚率は 1.7% であった。

B49. 転座染色体保因者 46,XX,t(10q-,20p+) の遺伝相談例：斎藤 勝・竹内 泉・河村 豊・藤丸純一・品川信良 (弘前大・産婦人) . Genetic Counseling for a Case of Reciprocal Translocation 46,XX,t(10q-,20p+): Masaru SAITOH, Yutaka KAWAMURA, Junichi FUJIMARU and Shinryo SHINAGAWA (Dept. Obstet. Gynecol., Hirosaki Univ. Sch. Med., Hirosaki)

染色体異常児分娩の既往があり、流産等を繰り返す患者で染色体分析の結果、46,XX,t(10q-,20p+) と判明した妊婦の遺伝相談を経験したので報告する。

相談者は 29 歳の主婦で、22 歳に健康な男性と結婚し、23 歳から 29 歳まで 6 回妊娠し、2 回の自然分娩、4 回の自然流産を経験している。2 回の分娩は妊娠 10 カ月で自然分娩しており、また、4 回の自然流産はいずれも妊娠 3 カ月以内に流産してしまっている。2 回の自然分娩の第 1 子はとくに異常を認めなかった。しかし第 2 子は妊娠 38 週で分娩したが、1,980 g の低出生体重児で呼吸異常、奇形等を認め、小児科で現在も入院管理しており、満 2 歳半に達したが、体重は 3,500 g くらいで、発育は非常に不良である。この児の染色体分析では、母親由来と思われる 46,XY,20p+ で 10q トリソミー症候群と考えられた。

相談者は現在妊娠 9 カ月を経過しており、超音波断層法等による胎児発育は順調であり、また、著明な奇形等も認められない。

なお、妊娠 4 カ月の時点で羊水診断も施行した。

B50. 異常児分娩既往の遺伝相談について：森田良子・香山文美・佐藤孝道・水野正彦・坂元正一 (東大・医・産婦人) . Genetic Counseling for the Clients with Previous Fetal Abnormality: Y. MORITA, F. KAYAMA, K. SATO, M. MIZUNO and S. SAKAMOTO (Dept. Obstet. Gynecol., Univ. Tokyo, Tokyo)

産婦人科での遺伝外来の特徴の一つは、異常児分娩既往を主訴とした受診者が多いことであろう。今回、われわれは、昭和 51 年から 56 年までの 6 年間に異常児分娩既往を主訴として東大産婦人科遺伝外来を受診した 325 例を検討し、若干の知見を得たので報告する。

1) 325 例の異常児の内訳はダウン症が 171 例と過半数を占め、その他の染色体異常児 30 例、奇形 64 例、先天代謝異常 18 例、伴性劣性遺伝性疾患 7 例、その他 35 例であった。当科は胎児診断を行っている施設であるから、それを目的として他科より紹介されたクライアントも多く、80% に及ぶ。また、これらの症例では一次的遺伝相談はすでに終了していると考えてよく、とくに染色体異常では妊娠してから訪れるものが 90% に及ぶ。一方、奇形児分娩既往者では、妊娠してから来院したものは約 1/3 と少なく、多くは妊娠の可否を求めて受診していた。2) 全症例の 56%, 181 例に胎児診断が施行され、10 例に異常が診断された。3) 異常児分娩既往者では、初回妊娠以前に遺伝相談を行うことによって異常児の出産を予測できたかどうかが問題となるが、今回の症例では家族歴から異常を予測しうる症例はみられなかった。4) 異常児のなかには、児が死亡しているものもあり、診断が不確実なまま遺伝相談を行わなければならない場合があり問題となる。新生児期における先天異常の診断と取り扱い上の問題点が示唆される。

B51. わが国の大学病院における先天異常外来の現状と問題点：木田盈四郎・上原真理子（帝京大・小児）. Present Condition and Problems on the Clinic for Congenital Anomalous Patients, Belong to University Hospitals and Medical College: M. KIDA and M. UEHARA (Dept. Pediat., Teikyo Univ. Sch. Med., Tokyo)

遺伝病，染色体異常，先天奇形患者が，全国 80 の大学病院小児科外来でどのように取り扱われているか，2 回にわたって調査した．1 回目は往復ハガキを用いたが，回答は 73.8% (59/80) で，そのうち，先天異常に関する特殊外来を設けているのは 52.8% (31/59) で，院内で染色体検査をしているところは 62.7% (37/59) であった（児臨，Vol. 35, pp. 651-659, 昭 57 年）．2 回目は調査用紙を用い，回答は 67.5% (54/80) で，特殊外来としては，遺伝相談と染色体外来は 28 (51.9%)，遺伝外来 22 (40.7%)，先天異常外来 21 (38.9%) であった．

現状に対する問題としては，専門医が育ちにくいこと 30 (55.6%)．遺伝相談を行うための困難な問題としては，医療体制のなかの限界 20 (37.0%)，患者診断の困難さ 19 (35.2%) であり，さらに，日常診療で困難を感じている疾患は，多発奇形 50 (92.6%)，先天代謝異常 47 (87.0%)，遺伝病 46 (85.2%)，染色体異常 43 (79.6%) であった．また，「遺伝病専門医」に必要な条件は，生化学・神経・染色体などの研究が 48 (88.9%)，国内外の人類遺伝学研究施設での研修 46 (85.2%)，臨床経験 46 (85.2%)，であった．現状を打開するために必要のこととしては，各大学医学部に人類遺伝学の講座をつくる 35 (64.8%)，遺伝相談ネットワークシステムの充実 35 (64.5%)，全国に数箇所先天異常モニタリングセンターをつくる 32 (59.3%) が目立った．

B52. A Family with Blue Rubber Bleb Naevus Syndrome: Kotaro YAMAOKA, Tatsuro KISU, Hisao MORI, Tsuneko YAMAGUCHI, Terutsugu NAKAMA and Takeharu HISATSUGU (Dept. Gastroenterol., Saga Med. Sch., Saga)

A clinical study was conducted on a family with Blue Rubber Bleb Naevus syndrome, and some genetic analysis was done as well. A propositus aged 32 years gave a history of upper abdominal pain lasting for a few months. Physical examination was normal except for tenderness in the right hypochondriac region. Survey of the skin demonstrated multiple blue rubber bleb naevi, varying in size from 1 mm to 15 mm in diameter, on the lips, neck, palms, soles and pubic region. A skin biopsy revealed that the nodule was a carvenous hemangioma. Upper GI tract series with barium contrast and endoscopy showed eight bluish angiomatous lesions from the esophagus to the gastric antrum. His past history included resected sigmoid colon after melena and artificial anus 15 years ago, and excised hemangioma of the pubic region 8 years ago.

The propositus was diagnosed as having cholelithiasis, incarcerated gall stone at the cystic duct, by abdominal echogram and drip infusion cholecystography. When a cholecystectomy was performed, multiple blue rubber nipples were found on the surface of the liver, small bowel, colon and retroperitoneum.

The father of the propositus, a brother, a sister, an uncle, an aunt and a grandfather together with 17 other family members on his paternal side were affected by multiple skin

angiomatous lesions. The family pedigree of 24 members so affected out of 73 strongly suggests that the condition was inherited as an autosomal dominant abnormality; there was no evidence of sex linkage. HLA-A, B, C, and DR antigens were examined in 8 members, but the HLA system did not seem to play a role in Blue Rubber Bleb Naevus syndrome.

B53. A Case Report of the Poland-Möbius Syndrome: Hideo TAKI, Satoshi KUSUDA, Yukinobu OHSASA, Yutaka HASE and Tsuneo TSURUHARA (1st Div. Pediat., Child. Med. Cent. Osaka City, Osaka)

Case report

S.M., this 9-month-old male infant was admitted to our hospital because of cyanosis and multiple congenital abnormalities on the 1st day of life. The patient was delivered by caesarean section during the 41st week of gestation because of severe EPH gestosis. The mother denied usage of drugs, exposure to X-rays or toxins during pregnancy. Maternal polyhydramnion was recognized. Apgar scores were 5 and 8 at 1 and 5 min. The birth weight was 3,464 g. There was no family history of congenital birth defects or neurological disease. The parents were not related.

Physical examination. He had the following congenital abnormalities: (1) fixed expression less facies with inability of the eyes to move on lateral or medial gaze, (2) inability to close the lids completely, (3) downward slant, (4) micrognathia and hypoplastic mandibles, (5) aplasia of the sternal portion of the left pectoralis major muscle, (6) aplasia of the left pectoralis minor muscle, (7) absence of the left nipple, (8) left-hand acromicria with soft tissue syndactyly and brachydactyly of the second, third, fourth and fifth fingers, (9) partial ninth and tenth nerve palsies with a weak gag reflex and disturbed swallowing, (10) mild VSD. His hearing was slightly disturbed.

Radiographic study. The chest and skull films were normal. The length of bil. humeri, radii and ulnae was same. In the left hand, there was brachydactyly with hypoplasia or absence of first or second phalanges or index, middle, and ring finger, and absence of phalanges of the fifth finger.

Chromosomes. Conventional and G banding analysis showed a normal 46,XY karyotype. Developmental milestones were severely delayed. (His head control was not steady at the month of nine.)

- B54. 3 Cases with Abnormalities of the Tongue and the Limbs: Takuya IKEDA, Asao YARA, Kiyotaka HIRAYAMA** (Dept. Pediat., Ryukyu Univ., Okinawa), **Kenji NARITOMI, Tamotu TERAWAKI** (Dept. Pediat., Kagoshima Univ., Kagoshima), **Shozo OHDO and Harumiti MADOKORO** (Dept. Pediat., Miyazaki Med. Coll., Miyazaki)

Möbius syndrome, glossopalatine ankylosis, aglossia-adactylia syndrome and Poland-Möbius syndrome are known as the syndromes with microglossia and congenital malformations of the extremities. Among these four syndromes only Möbius syndrome has been considerably reported in Japan. But the others have not ever been reported as far as we could search in Japan. We reported here three cases with microglossia and congenital malformations of digits. Case 1 was a 5-month-old boy and diagnosed as typical aglossia-adactylia syndrome with a band between left upper palate and left lower alveolar ridge. Case 2 was a 7-day-old girl and diagnosed as glossopalatine ankylosis with digital hypoplasia. Case 3 was a 1-month-old boy and diagnosed as Möbius syndrome with hypoplasia and syndactyly of fingers and transverse amputation of the lower limbs. All of these three cases were sporadic and had normal chromosomal karyotypes.

- B55. Familial Occurrence of Endocardial Cushion Defect: Yukio SATOW, Kiyokazu OHMAE and Naomasa OKAMOTO** (Dept. Genetocopathol., Res. Inst. Nucl. Med. Biol., Hiroshima Univ., Hiroshima)

At the last congress of this society we presented that some cases of the monozygotic twins showed more often the tendency of the left QRS axis deviation and the notches on R-waves in the electrocardiograms with age than those in control group.

The left QRS axis deviation are found in some cases of both congenital and aquired heart diseases. As to how much it deviates, it differs much from case to case and is not always consistent with the findings of the outer figures of hearts.

The proband of this case is a 33-year-old male. He was diagnosed clinically as an incomplete endocardial cushion defect (ostium primum defect), which was proved to be true by the operation. His QRS axis was normal ($+77^\circ$) probably because the mitral valve was normal. His eldest sister was also diagnosed clinically as an incomplete endocardial cushion defect (ostium primum defect). Her QRS axis was -79° . She died of complete A-V block at the age of 38. His elder sister died suddenly of congenital heart disease at the age of 21, though she had not suffered from cyanosis since her childhood. His parents are first cousins each other. They both showed mild hypertension and suspected hypertrophic myocardosis. Their QRS axes deviate to the left remarkably, showing -84° and -33° , respectively, which is not consistent with the above clinical findings, but might be presumably caused by the abnormal development in the conduction system.

Further investigation should be carried out to find the causes of the left axis deviation

in an electrocardiogram as it might be one of the indexes of the genetically controlled congenital heart diseases.

B56. Pycnodysostosis の 1 例：宮城 茂・仲田教子・樫田 豊・石飛和幸（鳥取大・医・三内）. A Case Report of Pycnodysostosis: S. MIYAGI, N. NAKADA, Y. HITSUDA and K. ISHITOBI (Dept. Intern. Med., Tottori Univ., Yonago)

Pycnodysostosis は、青池が 1954 年に dysostosis generalisata と osteopetrosis の合併例として最初に報告して以来、本邦では現在までに 50 例以上の報告がある。われわれは典型的な本症の 1 例を報告し、またこれまでの本邦報告例 46 例とを併せて検討した。症例：9 歳男子。主訴：低身長。家族歴：血族結婚（－） 現症：身長 117 cm (-2.7σ) の均衡型小人症、特有の鳥状顔貌、小顎症、大小泉門開存、両側眼球突出、歯列不整、短指症、両側指趾 overriding nail. X 線所見：骨格系全体の骨硬化像、頭蓋泉門縫合開存、指趾末節骨の融解像、上下顎骨の形成不全。骨塩量 0.649 g/cm^2 と増加。染色体 46, XY.

本症例を加えた 47 例の報告では兄弟例が 7 家系 17 人、父子が 1 家系 2 人、ふたいとこ 1 家系 3 人の同胞発生が認められ、散発例は 25 例であった。両親の血族結婚が 71% に認められ、いとこ結婚 52%, いとこ半婚 4.5%, ふたいとこ婚 4.5%, 不明 10% であった。性別では男性 17 例、女性 30 例と女性に多く、診断時の身長は全例平均身長より低く $-1.0\sigma \sim -6.5\sigma$ (平均 $-3.3 + 1.3\sigma$) の低身長を示した。染色体検査例 8 例はすべて正常。全例に骨硬化像を認め、泉門縫合開存は 98% に、下顎角減少および消失は 96% に、末節骨の形成不全は 97% に、易骨折性は 96% の症例に認められた。

B57. A Clinicogenetic Study of Spontaneous Occlusion of the Circle of Willis: Mikako KAWAI, Makiko OSAWA and Yukio FUKUYAMA (Dept. Pediat., Tokyo Women's Med. Coll., Tokyo)

The etiology of moyamoya disease is still completely unknown. Through a review of the available literature, the authors compiled familial cases involving 35 families. As no clinicogenetic study of this disease has been undertaken in the past, we performed this study.

Materials and methods. A total of 187 affected families was collected by sending questionnaires concerning family histories to hospitals from which cases had been pooled in the research center for this disease (Keio University) in Japan.

Results. The male : female ratio of the probands was 1 : 1.8. No consanguinity was observed. The breakdown of probands by age showed that 63% were pediatric cases. Familial occurrence was observed in 11 families (5.9%), of which the parent and child were afflicted in two, the siblings in eight and the relatives in one. Twins were seen in three (1.6%). A pair of MZ twins was concordant as to the disease. The recurrence rate in siblings was 2.4% (with the pediatric group, 4.0%; with the adult group, 1.1%). We estimated that the lowest limit of the population frequency of moyamoya disease (P) was

0.07%. Thus, our figures were 30–60 times higher than P. Our percent frequency in sibs (2.4%) approximated $\sqrt{p}$ (=2.6%). These findings suggest multifactorial inheritance.

B58. Gilles de la Tourette Syndrome-Risk Factors and Familial Clustering: Kiyotaro

KONDO (Tokyo Metropolitan Inst. Neurosci., Tokyo) and **Yoshiko NOMURA**
(Segawa Neurol. Clin. Children, Tokyo)

Gilles de la Tourette syndrome (GT) manifests multiple tics, impulsive behaviors, obscurities in 2–15 years of age, especially in boys. Psychogenic, pre- or perinatal and genetic etiologies are suspected. 32 boys and 11 girls with GT were matched with 43 boys with Duchenne muscular dystrophy (DMD). No difference was observed between GT and DMD in maternal diseases, outcomes or complications of the foregoing as well as the present pregnancies, perinatal disorders, and developmental milestones before developing the diseases. No birth order effect was observed in GT. Mental variables were difficult to evaluate, but showed no gross difference. Eliminating one sibling of GT proband who was also a proband, 7 of 42 probands (17%) had one or more affected relatives. Of a total of 428 known relatives only 9 (2%) evidenced GT or GT-like conditions, male cases being predominant. Risks decreased as the blood relations to the proband decreased. Only one parent pair was inbred. No mendelian pattern was evident in these pedigree materials. This study, the first case-control evaluation of GT, gave no support for various suspected risk factors, but could not establish the mechanism of its familial onset.

B59. Studies of Genetic Markers in Blood in the Arao Focus of Familial Amyloid

Polyneuropathy: S. SAKODA, T. SUZUKI, S. HIGA, M. UEJI, S. KISHIMOTO
(3rd Dept. Intern. Med., Osaka Univ. Hosp., Osaka), **A. HAYASHI, Y. WADA**
(Osaka Med. Cent. Res. Intern., Matern. Child Health, Osaka), **A. NAKAJIMA**
(Nakajima Med. Clin., Kumamoto), **Y. TAKABA** (Arao City Hosp., Kumamoto), **H. MATSUMOTO, T. MIYAZAKI** (Dept. Legal Med., Osaka Med. Coll., Takatsuki), **T. SASAZUKI, K. NISHIMURA, M. EGAMI** (Dept. Hum. Genet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo), **K. OMOTO and K. TOKUNAGA** (Dept. Anthropol., Univ. Tokyo, Tokyo)

Blood samples from 21 patients with familial amyloid polyneuropathy (FAP) and their 81 family members among 7 affected families in Arao were tested for 9 blood group systems, 8 serum proteins, 12 red cell enzymes and HLA. The existence of two relatively rare variants, *i.e.* group specific component J and phosphoglucomutase 7 in 3 families suggested that 3 of 7 families have a common ancestor. Phenotype AB in ABO blood group, phenotype I in haptoglobin system and genotype M₂ in protease inhibitor system were not seen, and phenotype a/a in Kidd group were only one in patients. But it remains uncertain whether these findings represent linkage between FAP and these genetic markers. Phenotype

types peculiar to Caucasian were not found in this study, so it couldn't support the hypothesis that FAP in Japan is originated in Portugal.

B60. The First Report of Joseph Disease in Japan. Neurological Disease Inherited among the Portuguese: Tetsuo SAKAI (Dept. Neurol., Natl. Akasaka Hosp., Fukuoka), Michiya OHTA (Dept. Neuropathol., Neurol. Inst., Kyushu Univ., Fukuoka) and Hiroshi ISHINO (Dept. Psychiat., Shimane Med. Univ., Izumo)

Joseph disease has been thought to be a dominantly inherited neurological disease only among the Portuguese or Portuguese ancestry, and there are three phenotypes, clinically. We now report a Japanese family of Joseph disease residing in Beppu and Oita cities. The family involved 11 affected members (male 8, female 3) through four generations, indicating autosomal dominant inheritance. On examination at age 45, the proband man, having an ataxic gait since 40, showed normal mentality, lid retraction, ophthalmoplegia (especially, upward), horizontal and vertical nystagmus, faciolingual fasciculation, mild dystonia of the face and hands, marked spasticity of legs, generalized exaggerated tendon reflexes, marked ataxia of limbs and marked spastic-ataxic gait (type II, Joseph disease). Case 2, the elder brother of the proband, had ophthalmoplegia, nystagmus, pyramidal signs and limb ataxia at age 32, and died 6 years later. Neuropathologically, there is marked degeneration of the substantia nigra, dentate nuclei, pons, spinocerebellar tracts and anterior horn cells of the spinal cord. This family was confirmed to be Joseph disease. Case 3, 59-year-old male, had nystagmus, dysarthria, marked limb ataxia, abolished tendon reflexes in the legs, and peripheral sensory loss who was thought to be type III, Joseph disease.

Joseph disease has been reported since 1972 among the Portuguese in United States and Portugal (especially, Azores islands). This is the first report of pathologically proven Joseph disease in a non-Portuguese family.

C1. Counterstain-Enhanced Chromosome Banding Using Coriphosphin O, Quinacrine (QH, QM), Distamycin A (DMA), and Actinomycin D (AMD): Masafumi TANIWAKI, Kazuhiro NISHIDA, Junichi EDAGAWA, Yoshiaki SONODA, Shinichi MISAWA, Tatsuo ABE and Tatsuro TAKINO (Dept. Med., Kyoto Pref. Univ. Med., Kyoto)

Bone marrow cells from each patient with AML and CML, PHA-stimulated lymphocytes from a normal male, and an established cell line, BALL-1, were subjected to this experiments. Methods by which various banding were obtained are as follows: 1) Coriphosphin O/DMA staining. Slides were stained with coriphosphin O (0.1 mg/ml, in distilled water) for 20 min, rinsed 5 times with water, and stained with DMA (0.05 mg/ml, McIlvaine buffer pH 7.0) for 15 min. They were examined with V excitation. 2) Quinacrine/AMD staining. Slides were stained with QH (20 mg/ml), or QM (50 μ g/ml) for 5 min and then stained with AMD (0.01–0.1 mg/ml) for 15 min. They were examined with BV excitation. 3) 7-Amino-AMD/DAPI staining. Slides were stained with 7-NH₂-AMD (0.05 mg/ml) for 15 min and then stained with DAPI (0.2 μ g/ml) for 5 min. They were examined with UV excitation. Energy transfer-enhanced chromosome banding using coriphosphin O and DMA showed R-band pattern. A standard Ph¹ translocation in a CML-patient and complicated rearrangements in BALL-1 were clearly identified. Q-Bands (QFQ) with enhanced contrast were induced with QH/AMD staining. Use of 0.1 mg/ml AMD with quinacrine could quinacrine-bright polymorphic regions in a case of AML. 7-NH₂-AMD/DAPI induced QFH-type banding pattern with enhanced contrast in the prometaphase cells. The counterstain-enhanced chromosome banding reported here has potential application for studying chromosome polymorphisms and chromosome rearrangements, especially in neoplasms.

C2. High-Resolution G-Banded Patterns in Early Mitotic Chromosomes Obtained by the Ethidium Bromide Pretreatment Technique: Tatsuro IKEUCHI (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

The new standard nomenclature diagram for high-resolution banding of human chromosomes has recently been published (ISCN, 1981). To obtain an adequate number of early mitotic cells with elongated chromosomes which permit high-resolution banding, most investigators are employing the cell synchronization techniques by means of either methotrexate or excess thymidine. The ethidium bromide pretreatment technique (Ikeuchi & Sasaki, *Proc. Jpn. Acad.* **52**: 15, 1981) is more simple and reproducible for this purpose, but its principle is essentially different from that of the synchronization technique: it is based on the inhibitory effect of EB on mitotic chromosome condensation. However, one might argue that the EB-intercalation to chromosomal DNA leads to the distortion of normal chromosome condensation process, which in turn results in the modification of

chromosome banding patterns. Therefore, the refined banding patterns obtained by the EB technique were compared in full detail with the standard diagrams in ISCN (1981). I demonstrated here in the poster session 6 to 8 representatives of high quality, GTG-banded (600 to 850-band stages) and relatively straight chromosomes in each chromosome number (22 autosomes, X and Y chromosomes). The mitotic cells were derived from PHA-stimulated lymphocyte cultures (from 4 subjects) treated with EB (7 $\mu\text{g/ml}$) and colcemide (0.02 $\mu\text{g/ml}$) for 2 hr before harvest. It was shown that the overall banding profiles on highly elongated chromosomes obtained by this technique fairly corresponded to the ideograms in ISCN (1981). Thus, the reliability of the EB pretreatment technique was further established.

C3. Family Studies of Chromosomal Heterozygotes with Structural Rearrangements of Balanced Type-Determination of the Break Points by a High Resolution Banding
Method: Yoshiaki KODAMA and Akio A. AWA (RERF, Hiroshima)

Chromosome analysis was conducted on two families in whom balanced type structural chromosome abnormalities had been detected in the F children of A-bomb survivors and their controls in the course of the F cytogenetic screening at RERF. The purpose of this study was to determine the origin and break points of abnormal chromosomes using the conventional stain, G-, Q-, C-, N-banding techniques, and G and R banding methods (abbreviated hereafter as HrG and HrR) in combination with a high resolution banding technique. Family I: A pericentric inversion of chromosome 2 was demonstrated first in the second daughter of the family. An extended study of her parents, two sisters and a brother revealed that the father and third daughter also carried this abnormality. It was thus confirmed that the father was a carrier with a karyotype of $\text{inv}(2)(\text{p}11.2;\text{q}13)$ (lightly stained G-band) by HrG, and $\text{inv}(2)(\text{p}12;\text{q}14.1)$ (darkly stained G-band) by HrR. Family II: Reciprocal translocation of chromosomes 12 and 13 was noted in a daughter of the family. However, the karyotypes of her parents were normal. Various tests for biological parentage such as blood typing, showed that there was no evidence to preclude biological parentage. Therefore, this abnormality was judged to have been arisen *do novo* as a chromosomal mutation. The break points by HrG was determined to be $\text{t}(12;13)(\text{q}21.32;\text{q}12.3)$ (lightly stained G-band) or $\text{t}(12;13)(\text{q}21.33;\text{q}12.2)$ (darkly stained G-band) by HrR. There was a difference in the determination of break points between G- and R-methods but this difference suggests that break points are located close to the boundary between lightly and darkly stained G-bands. A further analysis for the precise identification of break points would require sequential application of G- and R-band stain on the same metaphases.

C4. Phenotype of 4p Monosomy in Two Siblings with Normal Karyotype: H. OMORI, H. KASHII (Pediat. Clin., Kinan Gen. Hosp., Tanabe), R. TSUKINO and M. KOIKE (Dept. Pediat., Wakayama Med. Coll., Wakayama)

Two siblings with normal male karyotypes by G-band, having strikingly similar phenotype of 4p monosomy, characterized by microcephalus, hypertelorism, bow eye-brows, prominent glabella, cleft lip and palate, congenital heart disease, hypospadias *etc.*, are presented.

They were both males and small-for-data, their birth weights 1,715 g, 1,920 g, respectively. Their mother had one normal female and two abortion formerly. They died by the hemorrhagic tendencies of unknown origin at 11th and 8th day after birth. Their parents also showed normal karyotypes. About 90% of 4p monosomy are suggested to be caused by '*de novo*' and only 5% or less by a parental translocation. The break point is in most cases restricted to 4p15 or 4p16, and cytogenetic identification is usually not difficult. However, in our cases, all of them showed normal karyotype.

Three hypotheses are suggested as the etiology of our cases, *i.e.*

- (1) a very fine deletion of the short arm of the No. 4 chromosome impossible to identify by usual methods
- (2) autosomal recessive or X-linked recessive disorders
- (3) heterogeneity of 4p monosomy

Among them "hypothesis (1)" is most feasible because of their clinical features, two affected siblings and another two abortions in their family, and in this case, it must be suggested to be inherited by a balanced translocation carrier.

If our view is true, this suggested that in the development of the clinical features of 4p monosomy, very small deleted segment of the distal portion of the short arm of the no. 4 chromosome is responsible.

C5. A Case of Distal Trisomy 4q with Cardio-Facial Syndrome: Hiroshi NAKAI, Masue IMAIZUMI, Yutaka IGARASHI, Yoshio YOSHIDA, Megumu OYAMADA, Kiyoko KURATA and Keiya TADA (Dept. Pediat., Tohoku Univ., Miyagi, Chest Surg., Iwate Pref. Cent. Hosp., Pediat., Kamaishi Citizen's Hosp., Iwate)

A female infant with a cardio-facial syndrome was reported. She was also a distal trisomy 4q due to a maternal translocation.

At her birth, her father was 30 year old and her mother was 29. She was born by normal delivery at 39 weeks of a gestational age. Her birth weight was 3,480 g. The baby was nourished by a naso-gastric tube for a feeding difficulty and had digitalization to congestive heart failure. Her clinical features were severe psychomotor retardation, asymmetrical cry (left unilateral facial muscle weakness), lt. palpebral ptosis, lt. strabismus, limitation of the left arm movement, lt. Obuihimo syndrome (peripheral circulatory insufficiency due to

compression of the brachial artery by Japanese Obuihimo band), lt. torticollis, lt. auditory dysfunction, bilateral single transverse palmar creases, tapering fingers, clinodactylies, high positioned axial triradii, the third interdigital loops, enlargement of cerebral ventricles. Her electroencephalogram was within normal range and no episodes of seizure was recorded.

Chromosome analyses showed the father 46,XY. But the mother was 46,XX,t(3;4)(p25;q25), and the patient was 46,XX,-3,+der(3), t(3;4)(p25;q25)mat.

Several chromosome abnormalities were reported in the patients with cardio-facial syndromes. Though there was no constancy as to group, G. Cayler (1969) suggested that the chromosome 3 was affected more than the others. The relation between a chromosome and an embryogenesis of the cervico-cardiac region is a problem to be resolved in future.

C6. A Case with an Interstitial Deletion of the Long Arm of Chromosome No. 5:

Tetsuo FUKUDA, Yasushi ITANI, Yoshimitsu FUKUSHIMA and Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

We report a case with interstitial 5q deletion and discuss 5q- syndrome as a clinical entity. The proband, a female infant, was born at term to 30y mother and 29y father after uneventful pregnancy and delivery. At 2 years when she was referred to us, her weight was 10.4 kg (-0.9 SD), length 81.1 cm (-1.6 SD), head circumference 46.3 cm (-0.9 SD). Physical examination revealed the following abnormalities: narrow forehead, frontal bossing, epicanthus, blepharophymosis, anteverted nostrils, wide nasal alae, thick lips, retromicrognathia, clinodactyly of 5th fingers and club feet (treated). Dermatoglyphics was abnormal: 7 whorls and 3 loops, simian creases (bil), hypothenar pattern (lt), radial exit of main line A (lt) and distal axial triradius (tr) (lt). IVP revealed a horse-shoe kidney. DQ was estimated at 89. Chromosome study by using high resolution G banding showed 46XX,del(15)(q15q22). Both parents had normal karyotypes. Although there were no typical phenotypes to 5q- syndrome in previously reported cases and the present case, the abnormalities such as mental retardation, short stature, narrow forehead, frontal bossing, epicanthus, flat nasal bridge, anteverted nostrils, retromicrognathia, malformed ears, club feet, kidney abnormality and abnormal dermatoglyphics (simian creases, increased whorls, and hypothenar pattern) were frequent. The common deleted region was the band 5q15. 5q- syndrome as clinical entity requires further accumulation of additional cases.

C7. A Patient with 6p Trisomy Phenotype with Chromosome 8q+: Yasuyuki A.

SUZUKI, Yasuhiko ONO, Akiyuki OGAWA (Dept. Pediat., Oita Med. Coll., Oita) and **Takayuki SATAKE** (Beppu Seishien, Oita)

We presented a case with peculiar face, psychomotor retardation with hydrocephalus. His chromosome was 46,XY,8q+, while his parents' chromosomes were normal by G-

band method.

His phenotypic feature was similar to the 6p trisomy: small stature, prominent forehead, short palpebral fissure, eye lid ptosis, bulbous nose, long philtrum, low set ear, small mouth and pointed chin. By high resolution band method, it was supported that his karyotype was 46,XY,-8,der(8)(6;8)(p22;q24).

His serum complements (C3, C4 and CH50) were normal. He had HLA Aw24(9), A11, B15, Cw1, Cw4. His father had HLA Aw24(9), A11, B11, Bw60(40), Cw3, Cw4. And his mother had HLA Aw24(9), Bw52(5), B15, Cw1.

Our case had macrocephaly caused from arrested hydrocephalus. And mild pulmonary stenosis was found by Echocardiography. These complications were not seen in other reported cases.

C8. Langer-Giedion Syndrome (LGS) (Tricho-Rhino-Phalangeal Syndrome Type II, Acrodysplasia with Exostoses, Giedion-Langer) and Interstitial 8q Deletion: Yoshimitsu FUKUSHIMA, Tetsuo FUKUDA, Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent.), Toshiro IZAWA and Kikuo KAMESHITA (Div. Orthoped., Kanagawa Child. Med. Cent., Yokohama)

Langer-Giedion syndrome is a disorder characterized by sparse hair, pear-shaped bulbous nose with tented alae, cone-shaped epiphysis, multiple cartilaginous exostoses and mental retardation. Although the cause of LGS was previously considered to be unknown, three cases with 8q deletion were recently reported. We report further two cases with LGS and interstitial 8q deletion. Case 1 was a 4y7m boy and Case 2 a 15y girl. Both patients had the typical features of LGS including short stature, mental retardation, peculiar facies characterized by sparse hair, broad eyebrows, pear-shaped bulbous nose with tented alae, long philtrum, thin lips and large and protruding ears, and skeletal anomalies with cone-shaped epiphyses, ivory epiphyses, and multiple exostoses. Osteotomy of exostoses was performed in case 2 because of limited joint movement. High-resolution chromosome analysis revealed the same abnormal karyotype with 46,XY or XX,del(8) (q24.11q24.13) in both patients. The results of three recent reports and the present two cases suggest that 8q deletion is a cause of LGS. Though there was no deleted segments common to all cases, three cases with high-resolution analysis showed the interstitial deletion of 8q 24.1 in common.

C9. A Case Report of Partial Deletion of Long Arm to Chromosome 11; del(11)(q21q23): Hideo TAKI, Satoshi KUSUDA, Yukinobu OHSASA, Yutaka HASE, Tsuneo TSURUHARA (1st Div. Pediat., Child. Med. Cent., Osaka City) and Akitomo YOSHIMURA (Dept. Anesthesia, Osaka City Univ., Osaka)

Since the first description of the 11q— syndrome by Jacobsen *et al.* (1973), 21 cases have been reported. We here report a further case.

Case report

Clinical findings. T.K., a 18-month-old boy was the first child of healthy unrelated parents (maternal age 24 years, paternal age 25 years). The pregnancy was uncomplicated and uneventful delivery occurred at 38 weeks' gestation. There was no radiation history and no miscarriages except an artificial abortion. Birth weight was 2,700 g, length 48 cm and head circumference 34 cm. The infant was transferred to our hospital on the 5th day of life because of weak cry and feeding problem. He presented multiple physical defects. There included hypertelorism, upward slanted palpebral fissures, blepharoptosis, right pupil atresia and ophthalmophthisis, left retinal dysplasia, high arched palate, cleft palate, micrognathia, short neck, arachnodactyly, carp shaped mouth, wide spaced nipples, dextrocardia, heterotaxia and supernumerary thoracic vertebrae. Neurological exam. disclosed general muscle hypotonia. He had frequent, recurrent infections.

Growth and development. His developmental milestones were severely delayed; Good head control-7 months, Sits alone-14 months. At the age of 17 months he weighed 8,000 g, was 76.4 cm long and his head circumference was 42.0 cm.

Laboratory investigation. Brain CT showed slight atrophy of the brain substance and enlargement of the ventricles.

Cytogenetic findings. The cytogenetic analysis revealed the karyotype: 46,XY,del(11)(q21q23). Both parents had normal karyotypes.

C10. A Case of 13q- Syndrome with Unusual Renal Anomaly: Ayako OKI, Tetsuo FUKUDA, Yoshimitsu FUKUSHIMA and Yoshikazu KUROKI (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

We report a case of 13q- syndrome with unusual renal anomaly and discuss about the importance of the terminal portion of 13q on ureteral budding. The case was a 1y3m-old female infant. She was born to 28y-old mother and 34y-old father at 41 weeks' gestation (BW 2,800 g, BH 48 cm, HC 29.5 cm). During pregnancy, there were an X-ray exposure (1st trimester), genital bleeding (3rd trimester) and poor fetal movement. When seen by us at 1y3m, she had typical features as 13q- syndrome including developmental delay (DQ 44), short stature 72.2 cm (-2 SD), microcephaly 42.5 cm (-2 SD), trigonocephaly, round face, hypertelorism, long palpebral fissures, pinched nose, protruding maxilla, micrognathia, malformed ears, anterior perineal anus, clinodactyly of 5th fingers and abnormal dermatoglyphics (bilateral distal triradius t' and t'', right extra interdigital triradius d' and abortive left interdigital triradius c). High resolution G-banding revealed that the karyotype of the proposita was 46,XX,del(13)(q32.3). Though karyotype of the parents were normal, marker study using Q-banding revealed that deleted chromosome 13 had been originated from an error at maternal meiosis. By urological examinations, crossed renal ectopia with fusion and left supernumerary hypoplastic kidney were noticed. The cause of

these anomalies is considered to be ureteral missbudding. In patients with terminal 13q deletion, renal hypoplasia is the most common renal anomaly in the literature. The terminal portion of 13q seems to be related to ureteral budding.

C11. A Case of Ring Chromosome 14: Kimiko NAKAGAWA (Dept. Pediat. Hematol./Oncol., Child. Med. Cent. Osaka City, Osaka), Tomoko HASHIMOTO, Hiromi SAKAMOTO, Hide-aki CHIYO (Dept. Genet., Hyogo Coll. Med., Nishinomiya), Yoshiyuki TAKUBO, Masahiro SAKO, Shozaburo KONISHI, Akira FUJIMORI and Giichi TSUJINO (Dept. Pediat. Hematol./Oncol., Child. Med. Cent. Osaka City, Osaka)

The clinical features of r(14) syndrome were characterized by severe psychomotor retardation with seizures and minor craniofacial dysmorphism. We presented a male infant without psychomotor retardation at the age of 14 months. He came to our observation at the age of 4 months because of hepato-splenomegaly and leukocytosis, and he was diagnosed as CML. His main clinical features were flat nasal bridge, micrognathia, and drooping mouth. Though he had been observed to have tonic-clonic convulsion since the age of 8 months, anti-epileptic medication brought a satisfactory result. EEG, CT scan, and optic fundi showed no remarkable changes. Chromosome studies using various materials including peripheral blood lymphocytes, bone marrow cells, and skin fibroblasts revealed that he had a karyotype of 46,XY,r(14)(p13q32.3).

Schimit *et al.* (1979) reported some clinical similarities between r(14) syndrome and ataxia telangiectasis. The skin fibroblasts of our patient revealed normal sensitivity to X-rays. The clinical features of our case were not typical except for the seizures which was considered to be the most common sign of r(14) syndrome. It may be very possible that this phenotype was correlated to the chromosomal condition of partial monosomy of q32.3 → qter segment.

C12. Trisomy 14 Mosaicism; Case Report and Review: Nobuyoshi OZAWA, Kan-ichi SOH, Shidai KYO, Shinji SATO, Akira YAJIMA, Masakuni SUZUKI (Dept. Obst. Gynec., Tohoku Univ., Sendai), Tatsuro IKEUCHI and Akira TONOMURA (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

Spontaneous abortuses with complete trisomy 14 were detected by Kajii *et al.* (1972). The first live born infant with trisomy 14 mosaicism was reported by Rethoré *et al.* (1975). So far, only four other mosaic cases analyzed by banding methods have been reported. We present a case of trisomy 14 mosaicism which showed a karyotype of mos. 46,XY/46,XY,-14,+(14q), and compare with five cases already reported. The clinical features were characterized by growth retardation, psychomotor delay, broad nose, high arched

palate, micrognathia, short neck, heart defect and undescendent testes. Autopsy revealed the following findings; hypoplasia of cerebellum; hypoplasia of thymus, left pericardial sac defect, ventricular septal defect, patent foramen ovale, patent ductus arteriosus, abnormal lobulation of left lung (three), alveolar dysplasia, solitary subcapsular cyst of liver, malrotation of intestine, hypoplasia of bilateral adrenal gland.

A total of 41 metaphases from peripheral lymphocyte cultures was analyzed from 2 blood samples. Twelve cells (29%) showed 46,-D,(Dq), and 29 cells (71%) showed normal 46,XY. Other samples transformed by EB virus were analyzed and showed 46,XY/46,XY,-14,+i(14q) by G,Q-banding method. The proportion of the trisomic cells transformed by EB virus showed a marked decrease through serial passage.

C13. Partial 15q Distal Trisomy: Naoki NOMOTO, Yuri MIYANOMAE, Akira YOSHIDA (Dept. Pediat., Kyoto City Child Welfare Cent., Kyoto), Osamu NAGAUCHI (Dept. Clin. Lab., Kyoto City Hosp., Kyoto), Sachiko TUJI and Naoko HAMAGASHIRA (Dept. Pediat., Kyoto 1st Red Cross Hosp., Kyoto)

We reported a 8-month-old female with peculiar features, poor feeding and developmental retardation, who was trisomic for the distal part of the long arm of chromosome 15.

Her clinical features included low birth weight, poor feeding, hypotonicity, developmental retardation, long and deformity of the head, protruding of the forehead and the occiput, long and deformity of the face, hypertelorism, antimongoloid slant, internal strabismus, flat nasal base, small mouth, high arched palate, micrognathia, low set ears, malformed ears, short neck, hematoma of bilateral sternocleidomastoid muscles, funnel breast, atrial septal defect, slight contracture of all fingers, and bilateral talipes varus. Her weight was 4,040 g, length 60.5 cm, head circumference 40.5 cm and chest circumference 37 cm.

The proband was the product of a full term pregnancy born to a 26-year-old woman (gravida I, para I). Her father was 31 year old. Her weight was 2,700 g, length 49 cm, head circumference 37.8 cm and chest circumference 33.4 cm at birth. All but the proband were phenotypically normal.

Dermatoglyphic study showed the following; both palmar axial triradii in t'' position, simian crease on the left and L²/O in the hypothenar areas bilaterally, urnal loops in all fingertip patterns, and arch tibial patterns in the hallucal areas bilaterally.

Using G-, Q-, R-banding methods, the mother's or grandmother's karyotype was 46,XX, t(15;16)(q22;p13), while that of the proband was 46,XX,-16,+der(16), t(15;16)(q22;p13) mat, and the father's or brother's karyotype was normal.

C14. A Case of Phenotypically Normal Male with Ring Chromosome 21: Hiromi SAKAMOTO, Hide-aki CHIYO, Tomoko HASHIMOTO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med.), Hidenori YABUMOTO, Fumihiko IKOMA (Dept. Urol., Hyogo Coll. Med.) and Sachiyo DOI (Dept. Clin. Lab., Hyogo Coll. Med., Nishinomiya)

R(21) syndrome is a well-known one with severe mental retardation and considerable fatality. We experienced a case with ring chromosome 21 that showed no clinical features of r(21) syndrome.

Patient was a 31-year-old male. He came to us with a clinical diagnosis of azoospermia. We found he had one small ring chromosome. His Y chromosome was decided as normal by QFQ banding. The ring chromosome was identified one derived from chromosome 21 by QFQ and GTG banding. His intelligence was within normal limits. His development showed no remarkable retardation. His WBC had HY antigen as much as normal males'. His RBC also showed normal SOD-1 activity (15.0 U/ml). His ring chromosome showed no nucleolus organizer regions. His karyotype was confirmed 46,XY,r(21)(p11.2 q22.3) by high resolution banding technique. We speculated his ring chromosome 21 lost only little portion.

Three cases of ring chromosome 21 with normal phenotype have been reported. One is a mother of a boy with Down syndrome (47,XY,+r(21)). The others are a normal woman and her healthy son, discovered in the course of prenatal karyotyping.

C15. Isodicentric Chromosome in a Woman with Gonadal Dysgenesis: Tomoko HASHIMOTO, Hiromi SAKAMOTO, Chiharu TAKADA, Hide-aki CHIYO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med.), Koji KAYAMA (Dept. Obstet. Genec., Hyogo Coll. Med.), Norimitsu OHTSUKA and Yoshie SUGAHARA (Dept. Clin. Lab., Hyogo Coll. Med., Nishinomiya)

The 24-year-old female was seen because of amenorrhea. Her pubic hair and axillary hair was scanty and genitalia was infantile. She had normal height (153 cm, -0.8 SD) and well developed breasts. Total finger ridge count was 90 and none of Turner phenotype was found.

Chromosomal analysis revealed her karyotype as follows; 45,X/46,X,psu dic(X) (pter→q25::q25→pter). The dicentric chromosomes were found about 60% of metaphases in peripheral blood and 80% in skin fibroblasts. The large or bipartite X chromatins were found in buccal smear of the patient. Though all the dicentric chromosomes showed late replication pattern by the ordinary RBG banding method, the two types of patterns could be distinguished. The one (33% of the metaphases) showed symmetrical replication pattern but another showed asymmetrical replication ones. Yu *et al.* (1980) reported that the dicentric chromosome with asymmetrical replication pattern might be due to inter-

chromosomal rearrangement between two X chromosomes. But chromosomal condition of mosaicism in our case suggest firmly the mechanism, in which break and reunion between the two chromatids of the long arm and a successive non-disjunction of the X-chromosomes in the first postzygotic cell division caused the mosaic karyotypes.

C16. XYYY Syndrome in One-Year-Old Male: Hiroshi SHIONO, Noriko TABATA, Jun-ichi AZUMI (Dept. Legal Med., Sapporo Med. Coll., Sapporo), Seiichi HIRANO and Shigetaka IMAMURA (Dept. Pediat., Hokkaido Ryoiku Cent., Sapporo)

This report concerns the finding of an XYYY karyotype in one-year-old male with mental retardation.

The proband was the first child born to healthy unrelated parents. At the time of birth, the father was 32-year-old and the mother 31. There was no history of drugs, infections, or radiation during the pregnancy. Birth weight was 3,340 g (gestation; 41w). Moderate mental retardation was noted: Controlled his head 5 months of age, walked alone at 25 months. At the time of 10 months after birth he has undergone an operation of PDA. At age of 15 months, he was admitted to examine developmental retardation. Physical examination revealed no characteristic features. Gonadotropins in the blood were normal (FSH; 19.5 mIU/ml, LH; 2.6 mIU/ml). Dermatoglyphic findings were not characteristic.

The karyotype of the proband was 48,XYYY.

C17. (Cancelled)

C18. A Case of Chronic Myelogenous Leukemia with a Variant Ph¹ Translocation in an XYY Male: Kazuma OHYASHIKI (Dept. Intern Med., Tokyo Med. Coll., Tokyo), Mitsuo OSHIMURA, Kohtaro YAMAMOTO, Akira TONOMURA (Dept. Cytogenet., Tokyo Med. Dent. Univ., Tokyo)

We encountered a male patient, 38-year-old Japanese with chronic myelogenous leukemia (CML), whose bone marrow and peripheral blood cells in chronic and blastic phases contained a complex type of variant Ph¹ translocation and an extra Y-chromosome, *i.e.*, 47,XYY, t(9;22;13)(q34;q11;q14) (See table). A karyotypic analysis of PHA-stimulated lymphocytes showed the constitutional karyotype to be 47,XYY. Thus, it is considered that CML with the variant Ph¹ translocation developed in an XYY male patient; in the literature, such a case has never been reported so far.

B-lymphocyte cell lines with the complex type of Ph¹ translocation were established by the procedure of Epstein-Barr virus transformation. The presence of the complex type Ph¹ translocation in the B-lymphocyte cell lines suggests that some of B-lymphocytes originate from a CML clone.

Table. Chromosome findings on cells from the bone marrow, peripheral blood and an established cell line.

Date of examination		Materials	Karyotypes	Clinical phase	No. of cells analyzed
9/24	'79	P.B.	47,XY,t(9q+;22q-;13q-)	C.P.	5
9/25	'81	B.M.	47,XY,t(9q+;22q-;13q-)	B.P.	20
12/20	'81	P.B.(-PHA)	47,XY,t(9q+;22q-;13q-)	B.P.	20
		P.B.(+PHA)	47,XY,t(9q+;22q-;13q-); 47,YYY in 19 cells		20
2/4	'82	B.M.	47,XY,t(9q+;22q-;13q-)	B.P.	20
3/16	'82	B-cell line	47,XY,t(9q+;22q-;13q-) 47,YYY in 41 cells		50

P.B., peripheral blood; B.M., bone marrow; B-cell line, established on February 15, 1982, when the patient was in a blastic phase of CML; C.P., chronic phase; B.P., blastic phase.

C19. Chromosome Abnormality in a Case with Benign Thyroid Adenoma *in vitro*:

Hachiro SHIMBA, Toshio HIRAOKA and Shozo IIDA (RERF, Hiroshima)

The present report deals with the results of chromosome analysis in a male case with benign thyroid adenoma. Tissue biopsies from both tumorous and normal sites removed from a 48-year-old male were digested with 0.2% collagenase to disperse the cells in suspension, which were then cultured in serum-supplemented RPMI culture medium.

Chromosomes of the tumorous site were examined twice; on the 20th and 27th days after initiation of culture, respectively, when the epithelial cells predominated in the culture. Chromosome slides were prepared routinely by the air-dry method with conventional Giemsa stain. After microscopy and photography for well-spread metaphases, the slides were destained and G-banded by the trypsin digestion method for further karyotype analysis.

In chromosome slides from the first preparation, polyploid cells predominated, among which a small proportion of cells with endoreduplication were occasionally seen, but diploid cells were rarely observed. In the second sample, polyploid cells decreased in frequency to approximately 17%.

Chromosome analysis showed that there were two abnormal chromosomes; an outstandingly large chromosome and a chromosome smaller than any of the G group chromosomes in every cell examined. G-banding study demonstrated that the large abnormal chromosome was derived from a duplication of a large part of the long arm of chromosome 1 occurring at the distal region of the short arm of chromosome 1. The small chromosome was assumed to be produced by a deficiency of a brightly fluorescing region of the long arm of the Y by Q-banding method. No Y chromatin was observed in the interphase cell nucleus. Aberrations involving chromosomes other than 1 and Y were seen randomly only in a few

cells.

Fibroblasts grown from the normal tissue biopsy showed no chromosome abnormality: the brightly stained Q-band in the long arm of the Y chromosome as well as the Y chromatin were present in the interphase nucleus.

C20. A Family of Basal Cell Nevus Syndrome Accompanied with a Marker Chromosome: Shigekazu SHIMAOKA (Dept. Oral Surg., Osaka Dent. Univ., Osaka), Hide-aki CHIYO, Hiromi SAKAMOTO, Tomoko HASHIMOTO, Jun-ichi FURUYAMA (Dept. Genet., Hyogo Coll. Med., Nishinomiya), Chuzo MORIMOTO, Rikiya SHIRASU (Dept. Oral Surg., Osaka Dent. Univ., Osaka), Toshihiro GOTO (Clin. Lab., Shionogi, Osaka), Keizo SAKAMOTO and Hiroshi ITOH (Dept. Neurosurg. and Dept. Pathol., Hyogo Child. Hosp., Kobe)

Basal cell nevus syndrome is one of relatively rare autosomal dominant disorders. Proposita was 10-year-old girl. Main clinical signs were 1) cleft lip and multiple mandibular cysts, 2) short metacarpal bones, 3) particular appearance with hypertelorism and broad nose. Her father had a similar appearance to the proposita and had multiple maxillary cysts which had been taken a surgical treatment at the age of 18. Both of them were diagnosed to be this syndrome. A brother of the proposita died at the age of 16 months of hydrocephaly and brain tumor (medulloblastoma). He was also suspected to be suffered from the same genetic disorder because of some clinical signs, for example, cleft lip and the particular appearance.

In cytogenetic studies, a small marker chromosome due to the short arm of D or G group chromosome were found in the proposita and her father by routine chromosome analysis. It was difficult to consider that the marker chromosome might have any phenotypic effects in the members of the family. But the possibility that the cytogenetic abnormality would be due to the same genetic condition which would be essential to manifest the various features of the syndrome may not be deniable.

C21. Isoelectric Focusing Studies of PGM₁ in Japanese, with a Report of the PGM₂ Variant: Tohru ITOH and Itsuro NISHIGAKI (Dept. Genet., Inst. Develop. Res., Aichi Pref. Colony, Kasugai)

The distribution of phosphoglucumutase-1 (PGM₁) phenotypes in human red cells was determined by isoelectric focusing on 1,237 Japanese samples. Frequencies of the four common alleles at the PGM₁ locus were found: PGM₁¹⁺ 0.6302, PGM₁¹⁻ 0.1306, PHM₁²⁺ 0.1681, and PGM₁²⁻ 0.0578. Comparing with data from other ethnic populations, these quantities in Japanese are quite similar to those of Caucasians, but different from those of Africans. In addition to the common alleles, the allele PGM₁⁷, which could be also differ-

entiated into two subtypes of PGM_1^{7+} and PGM_1^{7-} , was detected with a polymorphic proportion in this population (PGM_1^7 0.0133). However, there existed a great difference in occurrence between these two alleles of PGM_1^7 ($PGM_1^{7+} : PGM_1^{7-} = 10 : 1$). Although only 33 individuals with the allele PGM_1^7 were analyzed in this survey, an accurate ratio will be obtained by checking more samples with PGM_1^7 .

In this screening of the PGM system by isoelectric focusing a heterozygote with a rare allele of PGM_2 was detected. Family studies and family register checkings for the propositus showed the evidence of hereditary occurrence of this allele and all the ancestors of the propositus are Japanese. As its electrophoretic pattern was most likely to correspond to PGM_2 5-1, we tentatively designate this allele as PGM_2^{5J} until confirmed elsewhere. As to the Mongoloids, no electrophoretic variants of PGM_2 have been reported, excluding pre-Mongoloids and aboriginal islanders. The PGM_2 variant detected in this survey is supposed to be the first case of the variant from Mongoloids and Orientals.

C22. Isoelectric Focusing of Rare Variants of Human Phosphoglucomutase-1 (PGM_1) and Phosphoglucomutase-2 (PGM_2): Norio TAKAHASHI and Chiyoko SATOH (RERF, Hiroshima)

When human erythrocyte PGM_1 and PGM_2 are examined by starch gel electrophoresis (SGE), not only are these phenotypes polymorphic, but various types of rare variants also found. Kaneko *et al.* and Masunari *et al.* present at this meeting rare variants of PGM_1 (11 types) and PGM_2 (5 types) which were identified in our Hiroshima and Nagasaki samples. These variants were analyzed simultaneously by polyacrylamide gel thin layer isoelectric focusing (PAGIF). Samples used were the same as those used by Kaneko *et al.* and Masunari *et al.* on their starch gel electrophoretic studies. Most of these kinds of variants which had been differentiated by slight differences of mobilities on SGE also be completely isolated and identified with PAGIF as well. Specifically, PGM_2 9_{NGS} 2, a fresh mutant, was identified by PAGIF as a variant completely different from PGM_2 9_{NGS} 1 which is a genetic variant. And PAGIF also demonstrated that $PGM_2^{5NGS} 1$, heretofore believed to be single allele on SGE, can be classified into two alleles ($PGM_2^{5NGS} 1$ and $PGM_2^{5NGS} 2$). However, there was no difference in isoelectric point between PGM_1 4_{HIR} 2 and PGM_1 1+ which had been completely separated by SGE. Further, using PAGIF, it was not possible to observe bands for PGM_1 5_{HIR} 1 and PGM_2 2_{HIR} 1, probably because they overlapped with bands of other more common types. These cases indicate that the mobilities of some proteins on SGE do not necessarily reflect their isoelectric point. Further analysis is necessary to these points.

C23. Transferrin (Tf) Variant in Hiroshima and Nagasaki Residents (2): Mikio FUJITA, Yoshiko TANAKA, Yuko NAGAHATA, Jun-ichi ASAKAWA, Chiyoko SATOH and Howard B. HAMILTON (RERF, Hiroshima)

We are at present conducting a screening program using starch gel electrophoresis for transferrins in plasma specimens obtained from residents of Hiroshima and Nagasaki. Since our report at the 24th meeting of this society, 6,673 subjects have been newly examined and including the study sample reported by Ferrell *et al.*,¹⁾ screening has been completed on a total of 19,770 subjects. When detected variants were reciprocally compared using polyacrylamide slab gel electrophoresis (PAGE) with a buffer of pH 8.4, 6 types of B variants and 7 types of D variants were detected. Further, when comparison of variants was made with the combination of PAGE with buffer solutions of pH 6.5 or 7.5 and polyacrylamide thin layer gel isoelectric focusing (PAGIF), the B variants proved to be of 7 types and D variants 9 types. This occurred because it became possible to differentiate B_{HIR} 6 from variant B_{HIR} 4, D_{HIR} 6 from variant D_{NGS} 1 and D_{NGS} 2 from variant D_{HIR} 4 which had shown the same migration rates when a single buffer of pH 8.4 was used. As in this case, it is difficult with PAGE in which buffer of only one type of pH is used, to detect slight differences in migration rates, on the other hand, PAGIF alone is inadequate to be used for comparison of all variants. Accordingly, we deem it necessary to make combined use of at least 3 types of techniques including PAGIF.

1) Ferrell *et al.* 1977. *Ann. Hum. Genet. Lond.* 40: 407-418.

C24. Genetic Polymorphism of White Blood Cells Glucose Dehydrogenase in Japanese:

Yoshio KERA, Kichihei YAMASAWA, Nao ITO and Setsuo KOMURA (Dept. Legal Med., Kyoto Pref. Univ., Med., Kyoto)

Glucose dehydrogenase [GDH, EC 1.1.1.47], a microsomal enzyme, is identical with hexose-6-phosphate dehydrogenase (H6PD). It oxidize glucose to δ -gluconolactone utilizing as a coenzyme either NAD or NADP. In mammalian tissues GDH is distinguishable from glucose-6-phosphate dehydrogenase [G6PD, EC 1.1.1.49] in several properties as subcellular localization, genetic trait, molecular weight, electrophoretic mobility, substrate specificity and immunochemical precipitation or inactivation. Recently, King and Cook (1981) have demonstrated genetic polymorphism of GDH using various tissue extract samples in a White British population.

In the present study, genetic polymorphism of GDH in white blood cells extracts from random adult Japanese ($n=322$) was investigated using polyacrylamide gel or agarose gel isoelectric focusing, followed by a specific zymogram technique. Three common phenotypes, which might correspond to GDH 1, GDH 2 and GDH 2-1 reported by King and Cook (1981), were observed at the pI s between pH 6.56-6.76 on the gel. No phenotypes with GDH³ component were detected so far. The allele frequency of *GDH*³ may be very

low among Japanese. The results of family study suggest that these phenotypes are inherited in the autosomal codominant trait. The allele frequencies of *GDH*¹ and *GDH*² in a Japanese population were 0.522 and 0.478, respectively.

C25. Study on Genetic Polymorphism of C3 in Japanese: Hiroaki NISHIMUKAI and Kichihei YAMASAWA (Dept. Legal Med., Kyoto Pref. Univ. Med., Kyoto)

Genetic polymorphism of C3 in Japanese population was studied. A total of 1,594 sera were obtained from unrelated healthy individuals, and reference sera were provided by Prof. Dr. Ch. Rittner, Univ. Bonn, FRG. C3 typing was carried out routinely by high voltage agarose gel electrophoresis. The C3 band zone was identified by the three methods: antigen-antibody crossed electrophoresis, immunofixation electrophoresis and hemolysis in gel assay for C3. One common (*C3*^S) and six rare variant genes were detected, and gene frequencies for these were as follows; *C3*^S=0.9944, *C3*^F=0.0006, *C3*^{S0.25}=0.0003, *C3*^{S0.02}=0.0003, *C3*^{F0.65}=0.0022 and *C3*^{F0.8}=0.0019 ($\chi^2=0.356$, $p>0.99$, df. 6). At least either *C3*^{F0.65} or *C3*^{F0.8} may be the second common gene in Japanese. From family studies, it was confirmed that C3 polymorphism was controlled by a pair of codominant genes at a single autosomal locus. Total complement hemolytic activities (CH50), C3/C3c protein concentrations and C3 hemolytic activities in sera with various C3 phenotypes were measured; these values were within normal ranges. It is suggested that variant C3 proteins may be functionally similar to common C3.

C26. IgG Heavy Chain Allotypes (Gm) in Autoimmune Disorders: H. MATSUMOTO, T. MIYAZAKI (Dept. Legal Med., Osaka Med. Sch., Takatsuki) and M. AKIZUKI (Dept. Intern. Med., Keio Univ., Tokyo)

Among specific immune response (Ir) genes, two main groups have been distinguished in humans and experimental animals; they are the genes linked to the MHC and those linked to the genes which control Ig allotypic determinants of IgG H chains. The association between certain HLA antigens and several diseases suggest the existence of pathogenic polygenes in linkage-disequilibrium with MHC complex. While only limited evidence is available for the association between Gm allotypes and the immune response to certain antigens in humans. We have studied the relationship between autoimmune disorders and Gm allotypes to examine the possible association between pathogenic factors and the Gm loci.

Serum samples from 343 unrelated normal individuals and a total of 454 from the patients with SLE and other disorders have been investigated for 14 Gm allotypes. The incidence of Gm a;-b0b35st haplotype was increased in patients with SLE ($\chi_{(1)}^2=9.34$, $p=0.02$), PSS ($\chi_{(1)}^2=9.79$, $p=0.02$), and MCTD ($\chi_{(1)}^2=13.25$, $p=0.004$). The total chi-square for

the four Gm haplotypes was significantly increased in SLE [patients with anti-RNP antibodies ($\chi_{(3)}^2=19.86$, $p<0.001$), and with anti-SSA antibodies ($\chi_{(3)}^2=25.64$, $p<0.001$). The association between the specific antinuclear antibodies and the Gm haplotypes was also found among the PM and PSS patients with antinuclear antibodies. Our data suggest the existence of Gm-associated pathogenic polygenes in certain autoimmune disorders.

C27. A Family with Eosinophil Autoregulation of Type I Allergic Bronchiale Asthma Induced with House Dusts: During Remission of Asthma Attack, Inhibitory Effect of Prostaglandin E_2 Released from Eosinophils on Histamine Release from Basophils and Mast Cells: Yoshio NISHIZAWA (Toyonaka Intern. Med. Clin., Osaka) and Atsushi MURAGUCHI (Lab. Clin. Invest., NIH, U.S.A.)

Eosinophils from the male patient (39 years old) with type I allergic bronchiale asthma induced with house dust increased and serum prostaglandin E_2 level elevated during remission of asthma attack. An inhibitor of histamine release was found to be associated with eosinophils from this patients. This eosinophil derived inhibitor (EDI) was released from eosinophil-rich fractions stimulated with the specific allergen *in vitro*. The active substance(s) in this EDI was determined to be a mixture of prostaglandin E_1 and E_2 by thin layer chromatography using with culture supernatant obtained from the specific allergen stimulated eosinophils. Indomethacin, an inhibitor of prostaglandin synthesis, specifically inhibited prostaglandins. Using by reverse haemolytic plaque assay with anti-prostaglandin E_2 -antibodies, it was directly showed that allergen stimulated eosinophils from the patients produced prostaglandin E_2 . These results suggests that increased eosinophil during remission of bronchial asthma produced prostaglandin $E_{1,2}$, and that as a result of binding of prostaglandin $E_{1,2}$ to these specific receptor on mast cells or basophils elevation of cyclic adenosine-monophosphate (c-AMP) in those cells inhibited release of anaphylactic substance from mast cells or basophils, *i.e.*, the eosinophil assumes to play a modulating part in the allergic inflammatory reaction by virtue of secreting a histamine release inhibitor such as prostaglandin $E_{1,2}$. Genetical analysis of the family showed an autosomal codominant type of the inheritance for this EDI productive gene.

C28. The Analysis of Pathogenesis in Patient with Hereditary Angioneuromatic Edema: Yoshio NISHIZAWA (Toyonaka Intern. Med. Clin., Osaka)

Hereditary angioneuromatic edema (HANE) that is thought to be due to deficient or abnormal activity of the inhibitor of the activated first component of complement (C1-INH) is transmitted as an autosomal dominant trait and assumed to be variant from described by Rosen *et al.* In this sense, new analysis of the genetical mechanism was expected. In the present study, it was analyzed the biochemical response to androgen or estrogen ther-

apy in patients with HANE. 1) HANE attacks decreased by administration of androgen. These were increased by administration of estrogen. 2) CI-INH protein and activity was also just the same change by these drug administration. 3) Hepatic cells obtained from normal dead body and/or HANE patient were cultured with serum obtained from a patient with complete CI-INH deficient. CI-INH protein in culture supernatant antincreased, when these cells from HANE patient was cultured added with androgen. CI-INH protein decreased in normal hepatic cell culture supernatant added with estrogen. 4) Using by indirect immunofluorescein stained method, CI-INH sport numbers were increased in patient's hepatic cells under the culture condition adding with androgen. These numbers were decreased in normal hepatic cells under the culture condition adding with estrogen. 5) These effects of androgen was observed in regardless of the nature of variant HANE types. In the conclusion, it suggests that androgen acts by increasing the levels of CI-INH protein and activity and that estrogen has opposite effect. There is a regulator gene about the structure gene of CI-INH in patients with HANE and this gene is transmitted as an autosomal dominant trait. The expression of this gene is promoted by androgen and repressed by estrogen.