シンポジウム 遺伝疫学へのアプローチ

Symposium. Approaches to Genetic Epidemiology

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遺伝疫学という学問領域の定義はまだ確立されていないが、遺伝学と疫学の両方のアプローチを使って、病気の原因を究明しようとする予防医学の新しい一分野であるといえよう。個々の遺伝疫学的研究でとられる方法論は、その具体的な目的あるいは形質によりさまざまである。本シンポジウムでは、現在わが国で行われている代表的な研究を方法論別に選び、5名の演者に各自の研究の目的と方法の概略、これまでに得られた成果と問題点、国外の研究状況、今後の見通しなどについて講演をお願いすることにした。

1. Approaches to Genetic Epidemiology Inborn Errors of Metabolism: Toshiaki OURA,
(Child. Med. Cent. Osaka City, Osaka)

There had been controversy with regard to the incidence of phenylketonuria (PKU) in Japan, because of a great discrepancy between the incidence obtained by statistical conjecture and the results obtained by several provisional newborn screenings summed to 100,000 tests until 1977.

Tanaka estimated the incidence of PKU in Japan at 1/10,000 to 1/15,000 using Dahlberg's formula on 66 family trees in 1966. Recent trend of marriage indicates that first-cousin mating has been remarkably decreasing, whereas that of first-and-half-cousin and second-cousin matings have essentially unchanged. Thus the statistical conjecture based on the rate of first-cousin mating has to be modified in consideration of new parameters of consanguinity.

Gamo, Kawabe and Oura reestimated in 1974 the gene frequency of PKU on 95 family trees using Fujita's formula that takes into consideration not only first-cousin mating but also the other consanguineous matings. The gene frequency was postulated 0.00518 ± 0.00104 . Accordingly the frequency of homozygote of PKU was 1/17,300 to 1/84,700, when we assume that coefficient of inbreeding was 0.000710. Those figures were found to be much lower than Tanaka's.

Japan started in 1977 nation-wide newborn screening for five inborn errors of metabolism, namely PKU, maple syrup urine disease, homocystinuria, histidinemia and galactosemia. At the end of March 1979, total number of tests reached 1,871,906 and the actual incidences of the diseases were elucidated.

Twenty six PKU patients were found with an incidence of 1/72,000 and unequal regional distribution. The PKU patients were most frequently seen in Kinki area with highest incidence of 1/26,650. Next to Kinki area, the incidence was 1/34,888 in Chubu-Hokuriku

area, followed by 1/55,763 in Kyushu area. Kanto area including Tokyo gave only one patient in spite of the biggest population occupying 1/3 of Japan's total population. The results suggest unequal distribution of PKU gene demonstrating more in the west-south and less in the east-north part of Japan.

Out of 26 PKU patients, at least one patient was proved to have malignant hyperphenylalaninemia due to aberrant bipterin metabolism, and some others may have so-called hyperphenylalaninemia. Careful follow up study is needed for the differential diagnosis.

The incidence of PKU in Japan is considerably low in comparison to that of Caucasian populations. World's overall incidence of PKU is roughly 1/11,000 according to Dr. Guthrie.

With regard to maple syrup urine disease, five patients were detected at the rate of 1/374,400 comparable to that of western countries. Three patients out of the five were born in Kyushu.

Homocystinuria was found in one infant in Kyushu, whereas hypermethioninemia was more frequently found, namely 1/112,000, by several causes.

One hundred and ninety one histidinemic infants were detected by the routine bacterial inhibition assay supplemented by thin layer chromatography for the detection of urocanic acid. The incidence of histidinemia was as high as 1/9,800, associated with a fairly even regional distribution in contrast with PKU. This figure is extraordinarily high in comparison to that of Caucasian populations which is only 1/30,356.

It has been well known that many individuals with histidinemia have normal mental performance, though some histidinemics have definite brain damage and/or speech difficulties. The indication of dietary treatment using low-histidine preparation remains to be investigated especially in Japan, where such a high incidence exists.

Galactosemia was detected by Beutler and partly concomitant Paigen-phage test at the rate of 1/156,000, which is approximately half of Caucasian's.

In conclusion, the newborn screening for the five inborn errors of metabolism disclosed the incidence and regional distribution of the diseases in Japan. This will contribute greatly not only to the prevention and treatment of the inborn metabolic disorders associated with brain damage but also to the scientific benefits in many aspects of genetics. Furthermore, Japan is the only country with big population, among non-white countries, which is performing nation-wide multiple newborn screening for inborn errors of metabolism. So the results so far obtained in Japan is an invaluable reference to other oriental countries in case they set up a similar survey in the hopefully near future.

2. 先天奇形サーベイランスについて：芦沢正見（公衛院・疫学）. *On Surveillance of Congenital Malformations. Masami ASHIZAWA* (Dept. Epidem., Inst. Publ. Health, Tokyo)

先天奇形サーベイランスは、一定の人口集団に発生する先天奇形の頻度を常時監視することによって、胎芽・胎児に加えられる未知の催奇形因子の導入・増加を早期に検知し、関係機関に警報を発することを第一の目標とするシステムであり、その公共性からみて、薬物・添加物等の化学物質、放射線等のエネルギー等のモニタリングと連関をもたせた行政システムの一環として運営されるべきであろう。

サーベイランスには、全出産を対象とする届出登録方式と特定の指定施設からの通報によるモニター方式とがあるが、いずれにせよ系統的に集積される情報は先天奇形の疫学研究上、また行政施策の上からも、他の方法では得られない資料を提供するものといえる。

前者の方式では登録される先天奇形は診断の比較的容易なものに限られるとか、限度があるものの、特定の先天奇形については、実発生頻度を時系列的に知ることができる。また登録後のレコードリンケージを効果的に行うことにより、出生後の年齢に応じたさまざまなリハビリテーションサービスの需要量を知ることができるなどの利便がある。

モニター方式による後者の方法は、モニターとしての施設の妥当性、診断基準の統一とその達成度の平準化、モニター情報の収集・解析、結果の配布計画など、事前に十分なチェックが必要となる。モニターだけの分岐はモニターによるバイアスがさけられないので、正確な発生頻度を表すものではないが、疫学研究の常道である、1) 流行の集団間比較研究、2) 患者一対照研究、3) コホート研究、4) 介入研究等には適している。

サリドマイド事件を機として、先天異常サーベイランスシステム設置の提案はすでに T.H. Ingalls と M.A. Klingberg により 1963 年になされたが、その後、各国で病産院グループ、地域または国レベルのモニタリングが拡がりつつあり、1974 年アメリカの National Foundation—March of Dimes の援助により、先天異常国際情報交換機構 (International Clearinghouse for Birth Defects Monitoring Systems) が設立され、現在 17 のモニタリングプログラム (21 か国) が加盟し、四半期報が回報され、分類、疫学緊急調査、複合奇形等の委員会も設けられている。

演者らの都内日赤 5 施設のサーベイランス研究班は 1978 年 4 月より上記の国際機構に加盟が認められたが、年間分娩 8,000 台というサイズなので、モニター組織の拡充が要望されている。発生頻度の有意増加の検定にはサイズが比較的小さくても可能の点に利点があるといわれる R. Chen のセット技法を検討中である。この一部は今年の日本先天異常学会に報告した。

なお本研究は昭和 50, 51 年度厚生省心身障害児療育研究班, 52, 53 年度厚生省心身障害予防遺伝研究班研究費の補助を受けた。

3. 神奈川県ふたごレジスター：松井一郎（神奈川こども医療センター・遺伝科）. *Newborn Twin Registry in Kanagawa Prefecture, Japan: Ichiro MATSUI*, (Kanagawa Child. Med. Cent., Yokohama)

昭和 50 年より、神奈川県全域をカバーするふたごレジスターを開始した。県下の 37 保健所で双生児（多生児）出生のさい、人口動態出生票・死産票を転写し、資料をこども医療センターに送付する（指定統計の目的外使用承認）。

資料を電算機に入力し年次別の双生児のデータバンクを作成した。このふたごファイルは、1) ふたごの統計と疫学、2) こども医療センター入院患児データバンクとの間で医療記録連結に使用される。

Fig. Twin register system in Kanagawa Prefecture, 1975~.

保健所送付資料の転写もれを防ぐため厚生省で編集された人口動態ファイルの多生児分を出力し、誤差の発生を防止した。全体のシステムは Fig. のごとくである。

1975, 76, 77 年の県内双生児発生率は 6.99%, 7.03%, 6.72%, で 1974 年の全国双生児頻度より高値を示していた。三生児の頻度もそれぞれ 0.065%, 0.136%, 0.089% で高値であった。

こども医療センター年間入院児は約 3,500 人で電算入力されており、各種統計表を出力している。先天異常 (ICD 740~759) は各年約 30% である。ふたごデータベースと入院患児データベースのあいだで医療記録連結を行った。照合の鍵として、生年、生月、生日、性、苗字の上 2 文字を用い、住所 (自治省コード)、双生児レジスター番号、病歴番号、ICD コード番号 (こども医療改変) をあわせて出力した。1975~1978 年の 4 年間で、病歴 14,369 件と双生児 4,394 人の連結で 269 名が出力され、正の連結 132 名、誤りの連結 137 名であった。双生児に先天異常を合併した貴重な症例を大量に検索できたことは、先天異常の成因研究に重要な手段を追加したことになる。

北欧と米国のふたごレジスターを紹介し、ふたご研究の国際的動向を論じた。

4. Biochemical Genetics Study of the Children of A-bomb Survivors: Chiyoko SATOH, Takeshi KAGEOKA, Kazuaki GORIKI, Jun-ichi ASAKAWA, Mikio FUJITA, Norio TAKAHASHI, H.B. HAMILTON (RERF, Hiroshima), Shotaro NERIISHI, Sadahisa KAWAMOTO, Ryuji HAZAMA (RERF, Nagasaki) and J.V. NEEL (Dept. Human Genet., Univ. Michi., Michi., U.S.A.)

After Muller reported the mutagenic effect of X-ray irradiation in *Drosophila* in 1927, others showed that ionizing radiation induces mutations in a number of animals and plants. At this Foundation since its inception as ABCC, later RERF, genetic studies of children born to A-bomb survivors have been a major part of the programs evaluating the late effects of the A-bombs.

The biochemical genetics study of children of A-bomb survivors was initiated in 1972, the ultimate purpose of which is to determine whether parental exposure has resulted in an increase in detectable mutations in the first generation offspring of the survivors. Using recently developed biochemical techniques it is now possible to detect gene mutations at the protein level: a variant protein found in a child, not present in parents, *i.e.*, not inherited from their parents, is a putative mutation.

Currently two screening methods are available to search for variant proteins; electrophoresis and enzyme activity measurement. Electrophoresis was the only method used at RERF until 1978. However, inasmuch as this method distinguishes a variant protein only when its net charge differs from that of the normal molecule, electrophoresis detects no more than 1/4 of all possible variants. Moreover, it is well known that a single electrophoretic variant, of which some abnormal hemoglobins are excellent examples, may conceal several different variant proteins. Many of the variants undetectable by electrophoresis have altered enzyme activity due to a conformational change of the molecule as a result of an amino acid substitution and K_m , V_{max} and the stability of the protein enzyme may be different from the normal. Furthermore, in view of some experimental evidence from offspring of irradiated mice with gene deletions, enzyme activity measurement appears to be complementary to electrophoresis.

By the end of 1978, blood samples from about 5,400 children of A-bomb survivors in Hiroshima and Nagasaki and 3,600 children of nonexposed parents were examined electrophoretically for 28 protein systems (4 proteins in plasma, hemoglobin A, A₂ and 22 erythrocyte enzymes) and 491 rare variants were found. Of these, family studies for 439 cases are in progress. Considerable differences between Hiroshima and Nagasaki in frequencies of variants of some of the proteins such as glucose phosphate isomerase, phosphoglucosyltransferase and transferrin are indications of some genetic differences between the populations of the two cities.

The measurement of enzyme activity was begun this year and 11 kinds of erythrocyte enzymes, mainly glycolytic are currently included in the screening program. Five hundred and eighteen children were examined for their enzyme activities by the end of August. Although precise characterization and family studies have not yet been completed, occurrence of activity variants seems to be higher than that of electrophoretic variants. At present it is premature to draw any conclusions concerning spontaneous mutation rate estimates or possible genetic effects of A-bomb radiation exposure in this population, since to date no more than half the number of individuals scheduled for study have so far been examined. However, when completed, this investigation may possibly yield estimates for the former and specific data on the latter.

5. The Central Registration System of Hereditary Gastrointestinal Polyposis. A Joint Study of Clinical and Genetic Epidemiological Approach: Joji UTSUNOMIYA, Takeo IWAMA and Masako TANIMURA (Second Dept. Surg. Polyposis Cent., Tokyo Med. Dent. Univ., Sch. Med., Tokyo)

The hereditary gastrointestinal polyposis include adenomatosis coli (familial polyposis coli, Gardner's syndrome and Turcot's syndrome), Peutz-Jeghers syndrome and juvenile polyposis coli.

They are rather rare in instance, but possess ultimate importances as a model for control of cancer by the high risk approach in epidemiological sense and for basic study in genetic aspects of carcinogenesis. They, adenomatosis in particular, are the condition of extremely high risk of cancer with an apparent Mendelian mode of transmission and with late manifestation, and produce large familial aggregation of patient.

Since the polyposis includes many heterogeneous condition of different etiology, specific clinicopathological consideration is required in ascertainment of the trait.

For practical purposes in prevention of large bowel cancer and for supplying the informations to basic study of genetic carcinogenesis, the polyposis center was established in our medical School in 1976.

The center's working system is composed by the proband survey, family survey and follow-up survey. Informations on the proband are collected through the following five sources: random inquires at hospitals, published case reports, autopsy records, voluntary referrals and Japanese society of large bowel cancer research. The trait of the registered case is carefully examined, classified, and eliminated. The precise pedigrees of the probands are constructed by using *koseki*, the national family register. The permanent coding system for family member ("the working pedigree") was devised both for epidemiological practice as well as for genetic research.

In the course of our study, there have been found "the fusion of pedigree" in 54 occasions. This effect frequently uncovered unknown part of many pedigrees. Presently, 397 families of adenomatosis, 122 Peutz-Jeghers syndrome, 193 multiple adenomas, 6 Juvenile polyposis, 55 non-polypotic familial colon cancer have been registered. On 306 families of adenomatosis (77% of the registered cases), family survey have been completed by examining on the informations of 4,535 family members. This confirmed presence of 848 affected which included 338 probands, 173 call up cases and 337 survey cases. One hundred and twelve members of 29 families of adenomatosis have been examined at the center, and 34 new patients were detected. These call up cases had large bowel cancer in much less frequency comparing with the proband cases. The member of the registered cases have been remarkably increased in recent years and the fact this increase is largely due to the increased member of the call up case demonstrates the effects of our activity of the center.

All the informations of the pedigree have been computerized and genetical analysis are

being carried out and producing many data on population genetics of the polyposes.

Blood sample and skin fibroblast are obtained from the patients and the other family member for use of linkage study, chromosome analysis and cellular biology. The recent advances in this field have revealed some specific characteristic of cultured fibroblast from the patient, and presented a hope for developing the marker detecting method of the polyposis gene cancer.