日本人類遺伝学会 第31回大会シンポジウム講演要旨

Abstracts of the Symposium, the 31st Annual Meeting of the Japan Society of Human Genetics

Symposium I. Human Genetics in Japan: The Status Quo and Future Desirable Status

SI-1. Future Prospect of Human Genetics: A Cytogeneticist's Point of View. Y. Nakagome (Natl. Child. Hosp., Child. Med. Res. Cent., Tokyo)

I have been in the field of human cytogenetics for some 26 years. In 1982, we decided that we should be fully familiar with the techniques of genetic engineering and should launch pertinent research projects. It was naturally based on my personal view on the future prospect of human genetics. First of all, it was hard to see how such problemes can be solved without using techniques of genetic engineering as mechanisms of X chromosome inactivation. The techniques are also potentially very useful in the diagnosis of a wide variety of inherited diseases through DNA polymorphism (RFLP). There was an additional reason: the projects were expected to attract more graduate students to our laboratory than that in the field of human cytogenetics. In the following part of this communication, I would like to discuss projects currently running in our laboratory with M. Yamada, Y. Nakahori, H. Kurosawa, A. Suzuki, N. Jinno and N. Suzuki.

Amplification of oncogenes in tumors in children: A total of 109 primary tumors of 25 different kinds were examined using 11 different oncogene probes including *fos, myc, N-myc, K-ras* and *sis*. Amplification of *N-myc* was observed in one out of 6 cases of rhabdomyosarcoma in addition to 4 cases of neuroblastoma. In the former, structural rearrangement involving 5′ side of the first exon of the *N-myc* gene was detected. Amplification of other oncogenes has not been detected in the examination of over 800 combinations.

Recessive mechanisms in the cause of tumors in children: In both Wilms' tumor (WT) and retinoblastoma (RB), an autosomal recessive mechanism of carcinogenesis has been detected. It was also found in osteosarcoma, rhabdomyosarcoma, hepatoblastoma and bladder carcinoma. Probes used were derived from either 11p or 13q. They were chosen because chromosomal deletion of either 11p or 13q was often associated with WT or RB. In nearly 50% of neuroblastoma, deletion of the short arm of no. 1 was detected. Using U1 small RNA gene as a probe, which is located within the 1p36 band, RFLP studies are in progress.

The use of RFLP probes in the diagnosis of genetic diseases: RFLP studies in the normal Japanese population are attempted. DNA extracted from 50 normal individuals were examined using different DNA probes. So far, 6 different probes were examined and in one of them only one of two types in the U.S. population (A1 : A2=85 : 15) were observed in all 60 Japanese (A1 : A2=100 : 0). In additional one probe, incidence was considerably different between the U.S. and Japanese populations (70 : 30 *vs.* 30 : 70). In an additional X-linked probe, what was reported as a constant band in the U.K. was very variable in Japanese although only 16 cases have been analyzed so far. These results indicate that there is a considerable racial difference in the RFLP pattern. A RFLP probe may not be useful unless it is tested in the normal Japanese population before they are used in the diagnosis of actual patients. Further studies are in progress.

Y-chromosome specific DNA: A Y-specific DNA fragment was cloned and its complete 3,564 bp sequence was determined. It was shown to be very useful in the detection of either a structurally abnormal Y or a normal Y in a form of mosaicism at a very low incidence through either *in situ* hybridization or Southern-blot analysis.

SI-2. 人類遺伝学の応用面の現状と問題点. 松田一郎 (熊本大・医・小児). **The Application of Human Genetics: The Present Status and Point at Issue.** Ichiro MATSUDA (Dept. Pediatr., Kumamoto Univ. Sch. Med., Kumamoto)

人類遺伝学の応用は主として，臨床遺伝学の領域で展開され，その内容も多岐にわたっている．単一遺伝子異常の疾患解析のみならず，成人病・薬物過敏症・アレルギーなど，単一遺伝子異常以外の疾患の解析についても，人類遺伝の基本的知識が応用されている．

一方，分子生物学の知識・手法が集積されるにつれ，その臨床応用は急速に拡大し，診断のみならず，遺伝子治療までその展望下におさめるに至っている．シンポジウムでは出生前診断と DNA 解析に的をしぼり，さらに今後の問題点に触れた．

1) 出生前診断：現状解析はアンケート調査によった．全国の大学産科・小児科およびその関連の施設 205 にアンケート用紙を送り，141 (産科 58, 小児科 83) の解答を得た．このうち行っているものは 64 (産科 37, 小児科 27)，現在行っていないが準備中および他の施設を積極的に紹介しているものが 10 で，合計 74 の施設が出生前診断に関与していた．内容は性決定・染色体検査，先天代謝異常症で，その症例数は次のようであった．

	1875年以前	1976～1980年	1981年以降
性決定	17	49	91
染色体検査	482	2,242	6,987
代謝異常症	31	119	205

2) 遺伝病の DNA 解析：応用面として，1) 特定の酵素の cDNA プローブを用いた変異遺伝子の検索，2) RFLPs を用いた保因者，出生前診断，3) 合成オリゴヌクレオチドを用いた (一塩基置

換の発見）遺伝子診断，4) RFLPs と linkage analysis による原因不明の遺伝病の DNA 解析を行っている我が国での現状を知る目的でアンケート調査を行った．

299 の研究施設（人類遺伝学会会員の所属している研究室，生化学教室など）に用紙を送り，197 の解答を得た．うち遺伝病の DNA 解析を行っている施設は 48 であった．内容をみると，1 つ以上の教室が手掛けている遺伝病としては，Lesch-Nyhan 症候群，OTC 欠損症，21-hydroxylase 欠損症，Duchenne 筋ジストロフィー，サラセミア，家族性アミロイドーシス，シトルリン血症で，他に 20 種の単一遺伝子障害，4 種の common disease，4 種の悪性腫瘍が含まれていた．

3) 今後の問題点：i）出生前診断への対応（諸外国ではこの問題の倫理問題は 10 数年前に十分に discussion されている）．ii）DNA 診断のための cDNA バンキングおよび情報の利用法．iii）遺伝病細胞のバンキング．iv）遺伝子治療の倫理問題（これは i）の問題と区別して行うべきものである）．以上について外国の実状も考慮に入れて今後，検討する必要がある．

SI-3. Human Genetics Teaching in Medical Schools in Japan: The Present Status and Point at Issue. **Hideo HAMAGUCHI** (Dept. Hum. Genet., Inst. Basic Med. Sci., Univ. Tsukuba, Ibaraki)

The rate of development of human genetics has steadily increased and the importance of instruction in human genetics in medical schools has become more obvious. In order to obtain information about instruction in human genetics in medical schools in Japan, the Committee for Research Promotion of Japanese Human Genetics sent 80 questionnaires to some members of the Japan Society of Human Genetics (professor or associate professor) or to the chairmen of the curriculum committees who were selected from each of 80 medical schools, in July, 1986. The present survey was the fifth among the surveys made by the Japan Society of Human Genetics; the similar surveys were made in 1962, 1970, 1972 and 1979. The present questionnaire was mainly intended to discover: 1) what courses are given, in what year, and for how many hours; 2) the content of the instruction; and 3) department or division of the individual(s) primarily responsible for the instruction.

Sixty-four questionnaires were returned and information about instruction in human genetics was obtained from 64 of the 80 medical schools (80%). Thirty-three schools (51%) provide a compulsory course in human genetics as an independent subject (29 schools) or as a course of an integrated curriculum (4 schools), with a mean of 26.1 hr. Eight schools (13%) provide instruction in human genetics systematically and compulsorily in a related subject, with a mean of 8.6 hr. The remaining 23 schools (36%) do not provide systematic instruction in human genetics. Many of the schools give the instruction in human genetics in the second year or the third year, that is, in preclinical school years but five schools provide the instruction in both the preclinical and clinical courses. In eleven schools (17%), courses include laboratory experiments ranging in length from 3 to 24 hr; the most popular experiment is the analysis of human chromosomes. The numbers of department or divi-

sion of the individual(s) primarily responsible for the instruction in human genetics are, in the order, genetics (human genetics, genetics or molecular genetics) in nine schools > legal medicine in seven schools > medicine in four schools, and public health and hygiene in four schools.

The results of the present survey suggest that the instruction in human genetics in medical schools has been partly improved in Japan. The most serious problem of human genetics teaching in medical schools in Japan, however, is that most of the medical schools do not have department or division of human genetics (or medical genetics or genetics).

SI-4. 普及と専門家養成. 池内達郎（東京医歯大・難研・細胞遺伝）. **Diffusion of Knowledge and Training for Specialists.** Tatsuro IKEUCHI (Dept. Cytogenet., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo)

知識の普及はさまざまなレベルの教育現場や啓蒙活動を通して行うことができようが，ここではとくに前者について言及した．大多数の国民にとっては，遺伝に関する基礎知識を得る唯一の教育の場は高等学校であろう．高校「生物」教科書の中で，人間の遺伝に関する項目の占める割合は，10年以前の1%以下から現在の約1.6%（5社の現行教科書しらべ）に増加し，分子遺伝病や代謝異常症，染色体異常症などの記載も散見されるようになった．しかし重要なことは，こうした疾患の単なる紹介だけでなく，多様な遺伝的変異の存在する人類集団（社会）についての正しい認識が必要で，教師の識見が養われる場としての例えば大学教育学部（生物専攻）での教育の在りようが問われるところである．

大学や各種専修学校（医療分野）における人類遺伝学関係の教育の現状を知ることを目的として，本学会会員（178名：評議員および他の学会員若干名）を対象にアンケート調査を実施した．大学においては，医学部以外の生命科学を対象とする学部および教養課程を対象とした（医学教育での現状は本シンポジウム I-3 で報告された）．回答者130名（回収率73%）のうち，大学の上記課程での教育担当者は25名（19%）のみで，国公私立併せて歯学部では13校，理学部（生物学）で10校，教育学部（保健体育関係）3校，他（教養，獣医，保健など）5校が挙げられた．理学部では独立科目として人類遺伝学の基礎分野が系統的に教えられ，歯学部では臨床分野も含めた関連科目の中で講義されている傾向が窺えたが，他の大多数の大学では生物科学関係の学部（理・歯・薬・農・教育など）学生にとって，人類遺伝学は未だ極めて馴染みの薄い領域と言わざるをえない．とくに人間の遺伝に関して多数の学問領域が広汎に発展している今日，基礎分野からの積極的な人材確保が望まれており，学生の関心を喚起させるための場（教育）が当然提供されねばならない．

専修学校での教育担当者は回答者の20%，臨検技師，看護婦，保健婦の養成校など計30校が挙げられた．しかしこの数値は全国の医療分野専修学校の3%弱にすぎない．医療の最前線にあるこれらパラメディカル関係者にとって人類遺伝学ないしは臨床遺伝学の高度な専門知識は必要不可欠で，ここでも系統的な教育システムを確立することが緊急な課題である．

このように，さまざまな教育の場で人間の遺伝学に関する教育が不足しているが，教官の確保が難しい状況にあることも事実である．究極的には，医科大学・医学部に人類遺伝学関連の専門講座が設置され，学部の枠を超えた人的交流が広く行われるべきである．このことは医学分野での教育や専門

家養成のためばかりでなく，国民の知識水準を引き上げるためにも，また医学関係以外に広く人材を求めるためにも極めて重要なことと言わねばならない．

SI-5. Promotions of the Human Genetics Research. Takashi IMAMURA (Natl. Inst. Genet., Mishima)

Advances in modern human genetics depend to no less an extent on individual enterprise and ingenuity in studying nature's experiments in human variation, but the rapid pace in the extension of our knowledge of the genetics of man has been stimulated by developments and introductions of new approaches and new techniques in cellular and molecular biology and in biochemistry and cytogenetics.

The complexity of the human genome is no longer beyond the grasp of molecular biology. The number of different gene products may be no more than 50,000 to 150,000, divided into a maximum of perhaps 10 to 15,000 families of related products, many of which may themselves be part of related super-families such as globins, immunoglobulins, major histocompatibility system, T-receptors and other lymphocyte surface molecules. This means that in the foreseeable future we may be able to identify the complete set of genetic functions that contributes to any phenotype. Mapping the human genome can make a major contribution to sorting out gene function relationships, as in the elucidation of significance of the specific translocations in leukemia and lymphoma, the identification of genetic changes in retinoblastoma or in Duchenne muscular dystrophy, the hunt for the cystic fibrosis gene or the Huntington disease gene, or simply using markers for screening or prenatal diagnosis. Polymorphic DNA probes now provide an essentially unlimited range of genetic markers for the search for linkage with well-defined Mendelian traits such as familial polyposis coli, or for the identification of genetic factors in less well-defined susceptibilities such as heart disease or cancer. The ultimate challenge is to unravel the genetic contributions of the molecular level to the determination of common traits, including the major chronic diseases, heart disease, autoimmune disease and cancer, as well as to normal variations, for example, physical features and behavior.

The boundaries of human genetics are indistinct and blurred. It is sometimes said that future progress in the sciences, particularly in those areas of importance for human health and welfare, will increasingly come from interdiciplinary fields by applying concepts and techniques from one field to another area. The success story of human and medical genetics can be cited as an example of such interdiciplinary research and applications. We must therefore continue to the lookout for methods from other field of the physical and natural sciences to be applied to our human genetic research area. Thus, human geneticists need to broaden the fields of interest to encompass other fields than those of medical interest alone. We need to attract more basic scientists and to establish more institutions where

the cooperative research on the human genetics could be promoted efficiently by those scientists with the joint appointment.

Symposium II. Molecular Genetical Appoach to Neuromuscular Diseases

SII-1. The Molecular Basis of Familial Amyloidotic Polyneuropathy. **Shuichiro MAEDA,[1] Shukuro ARAKI[2] and Kazunori SHIMADA[1]** ([1]Dept. Biochem., [2]1st Dept. Intern. Med., Kumamoto Univ. Med. Sch., Kumamoto)

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominantly inherited systemic amyloidosis, characterized by the extracellular deposition of fibrillar amyloid protein, which consists mainly of a variant prealbumin with a single amino acid substitution and contains a small amount of serum amyloid P (SAP) component. SAP has been detected in all types of amyloid deposits reported thus far, with the exception of the cerebral amyloid deposition associated with senile dementia of Alzheimer's type.

The clinical data of FAP in Kumamoto, Japan from 1967 to 1984 are summarized as follows. Ninety individuals with FAP were found among 734 members of 303 families in 8 pedigrees, and 50 cases were examined. The symptoms are first recognized when the patient is between 20 and 45 years of age, mean of 32 years. The sensory dominant mixed type peripheral neuropathy is usually manifest in the lower limbs. Dissociation of sensory impairment is common, with pain and temperature sensation most severely affected. The upper limbs are also frequently involved, starting a few years after the lower limbs. Autonomic nervous system involvement, such as sexual impotence, disturbance of gastrointestinal motility (alternating type of diarrhea and constipation), orthostatic hypotension and urinary incontinence are frequent. The disease is progressive and death follows after about 10–20 years.

We recently established the DNA diagnosis of FAP, using a cloned human prealbumin cDNA, restriction endonuclease(s) and Southern blot procedures. This approach clearly revealed a direct link between the prealbumin gene mutation and FAP; all the individuals with FAP, so far examined, are heterozygous for the prealbumin gene, carrying one normal and one mutant gene.

To study the control mechanism of prealbumin gene expression and to elucidate the molecular basis of FAP, we isolated a human prealbumin gene and determined its entire nucleotide sequence, including both 581 bp of the 5′- and 95 bp of the 3′-flanking sequences. The gene spans about 7.0 kb and consists of four exons and three introns. We also isolated a mutant prealbumin gene associated with FAP, and determined the sequence of the gene, including both the 5′- and 3′-flanking sequences, except for the internal portions of the

second and third introns. This sequence was in complete agreement with that of the normal one, except for a single-base substitution present in the codon for amino acid position 30. We also analyzed levels and distribution of prealbumin mRNAs in various tissues of control subjects and those of individuals with FAP, using the human prealbumin cDNA, as a probe. Prealbumin mRNAs were detected in the total RNAs from liver and choroid plexus of brain, but not in those from heart, thyroid gland, kidney, and brain. The levels of prealbumin mRNAs in the livers of control subjects and those of individuals with FAP were much the same. Because all the individuals with FAP, so far examined, were heterozygous for the prealbumin gene, the levels of the normal and mutant prealbumin mRNAs in the liver and the brain tissue containing choroid plexus were separately estimated and were found to be approximately equal.

The arguments that the mutant prealbumin gene is crucial to the pathogenesis of FAP are persuasive. However, as clinical symptoms of the dominantly inherited FAP appear only when the individual reaches adulthood and the fibrillar amyloid deposits in FAP consist of two different proteins, a variant prealbumin and SAP, a definite conclusion from the above data cannot be reached.

Transgenic mice provide the means to test directly the efficacy of the variant prealbumin and SAP *in vivo*. In an effort to construct mouse model systems for the FAP, we have isolated mouse prealbumin cDNA and genomic DNA clones, and determined their nucleotide sequences. Certain regions of their structures were revealed to be highly conserved in mice and humans. We also isolated complementary and genomic DNA clones corresponding to the human SAP mRNA and determined their sequences.

Once mouse model systems for the FAP become available, studies on them should yield pertinent data to elucidate the molecular basis of FAP. Moreover, these animals will be an appropriate model for biochemical studies on the treatment of FAP.

SII-2. Duchenne Muscular Dystrophy. Hideo SUGITA (Div. Neuromuscular Res., Natl. Inst. Neurosci., NCNP, Kodaira)

Duchenne muscular dystrophy (DMD) is one of the most common muscle-wasting diseases in childhood, characterized by a X-linked recessive trait. It occurs about once in every 3,000 to 4,000 male birth and one-third of these cases arise from new mutation, suggesting an abnormally high mutation rate, $5.0–10.6 \times 10^{-5}$. The muscle wasting progresses continuously, but no one has any idea why or how this occurs on molecular level. In this symposium, the recent progress of the molecular genetics on DMD is reviewed.

Two groups of investigators, using different strategies, are closing in on the gene for DMD: One is dealing with the female DMD with X-autosome translocation, and the other with the boy having the focal deletion of the X-chromosome spanning the DMD gene locus.

1. Duchenne or Becker muscular dystrophy females carrying X-autosome translocation. DMD is essentially the disease of the boys, but very rarely, females also suffer from the disease with the same clinical course as boys. Since Greenstein *et al.* reported the afflicted girl with X-autosome translocation, 5 cases have been reported in Japan and more than 10 cases in Western countries. All cases have an X-autosome translocation that is definitely *de novo* and involves an exchange point within band Xp21. In all cases, autosomes are different in each case; the normal X chromosome is late replicating and presumed to be inactive in all or most cells examined.

These findings give a strong support for the location of the DMD locus in the region of Xp21 and confirm the hypothesis that the translocation has disrupted the normal genetic activity of the DMD gene which together with the non-random inactivation of the normal X chromosome results in the full manifestation of the disease.

One female patient, studied by Verellen-Dumoulin *et al.*, had a translocation involving a block of ribosomal RNA genes of chromosome 21. Ray and his group used rRNA sequences as probes to clone the region spanning the translocation breakpoint. XJ-1.1 derived from X-chromosomal portion of the clone detects a RFLP which is closely linked to the DMD gene.

2. Deletions of the part of X-chromosome spanning the DMD gene locus. Recently several families were described in Japan in which the boys suffered from an apparently X-linked syndrome consiting of DMD, congenital adrenal hypoplasia, and glycerol kinase deficiency. Detailed cytogenetic analysis of an afflicted boy and his mother revealed the deletion of Xp21 region, suggesting that the deletion caused these diseases.

Francke and collaborators described the case BB, afflicted with DMD as well as chronic granulomatous disease, retinitis pigmentosa and McLeod syndrome with the small cytological deletion at Xp21. Using the chromosomal DNA from the patient BB, Kunkel and his collaborators identified 7 DNA clones that lie within the region defined by BB deletion. They reported that 5 of 57 unrelated males with DMD exhibited the deletion of the subclones of one of 7 clonal DNA segment, pERT87 (DXS164 lesion) and these new DNA segments will be useful in carrier detection and prenatal diagnosis of DMD.

Furthermore, the subclones 1, 8 and 15 of PERT87 were tested for deletion by hybridization against the DNA isolated from 1,201 cases with DMD and 145 cases with Becker muscular dystrophy (BMD). Of all DMD and BMD males tested, 6.5% showed deletion at the DXS164 locus. Twenty four out of 57 deletions showed the presence of some subclones but not others and most have been shown to have independent breakpoints within DXS164 locus. Remaining 33 deletions were larger than 137 kb, because entire cloned regions were not found to be present.

We are also examining the feasibility of these pERT87 subclones to detect DMD and BMD patientes and carriers in affected Japanese families.

The pattern of deletions imply that the genes responsible for DMD must lie on a very large segment of X-chromosomal DNA and DMD is heterogenous on DNA level.

SII-3. Molecular Mechanism of the Female Lesch-Nyhan Patient. **Nobuaki OGASAWARA** (Dept. Biochem., Inst. Develop. Res., Aichi Pref. Colony, Aichi)

Lesch-Nyhan disease is an X-linked recessive disorder characterized by hyperuricemia, physical and mental retardation, choreoathetosis, and compulsive self-mutilation. The disease is associated with absence of activity of an enzyme involved in purine metabolism, namely hypoxanthine guanine phosphoribosyl transferase (HPRT). HPRT is X-linked and because of inability of reproduction in Lesch-Nyhan patient, this syndrome occurs only in males. We have, however, an unusual case of a girl with Lesch-Nyhan syndrome.

The patient's karyotype was 46,XX and there was no abnormality such as translocation. Her mother is not heterozygous for a deficiency of HPRT. This is confirmed by following results: 1) there was no HPRT negative hair follicle; 2) the HPRT activity of mother's fibroblast was normal, while the activity of the known heterozygote was approximately half of the normal; 3) there was no 6-thioguanine resistant cells in fibroblast and T-lymphoblast from the mother; 4) conclusively, the mother has two HPRT gene, while HPRT gene on the patient's maternal X chromosome was toally deleted.

As a hypothesis of the genetic mechanisms of HPRT deficiency in this girl, the attractive is that her HPRT deficiency represents a mutation in a regulatory gene. If the girl is deficient in a regulatory gene, fusion of her cells with the cells from the male Lesch-Nyhan patient would furnish the regulatory gene and the fused heterokaryons would produce active enzyme. These experiments, however, showed that there was no complementary nature between the female and male patients with Lesch-Nyhan syndrome.

To elucidate the mechanism of HPRT deficiency in this female patient, Northern and Southern blots analyses were carried out. Northern analysis showed no mRNA in the patient's B-lymphoblast. Although the patient had two X chromosome, only one copy of HPRT gene was detectable by Southern analysis. The existence of restriction fragment length polymorphism has been described for DXS10, which was anonymous human X chromosome specific gene and was closely linked to HPRT gene. Southern analyses after *Taq*I digestion showed that the mother was 7 kb/3.5 kb homozygous, the father 5 kb/3.5 kb hemizygous and the patient 7 kb/5 kb/3.5 kb heterozygous. These results indicate that the patient has two X chromosomes, one from the mother and one from the father and the analyses of DXS10 polymorphism can identify the maternal or paternal origin of X chromosome.

To find out on which of the X chromosome the HPRT gene is totally deleted, the patient's fibroblast was fused with LTK$^-$, a derivative of the mouse L cell lacking thymidine kinase,

and cells were selected in HAT medium. Southern analyses of DXS10 and HPRT gene of the hybrid clones showed that the clone having the maternal X chromosome alone deleted totally human HPRT gene, but the clone having the paternal X chromosome had human HPRT gene.

There must be a mutation or a modification on the paternal X chromosome which is not detectable in Southern analysis yet results in non-functional gene, since Northern analysis showed no mRNA in the patient's lymphoblast. One clone having the maternal X chromosome alone expressed human glucose-6-phosphate dehydrogenase (G6PD) and phosphoglycerate kinase (PGK). Six independent clones having paternal X chromosome alone did not expressed human G6PD and PGK. These results indicate that the paternal X chromosome is always inactive and the maternal X chromosome always active. Thus, the most likely genetic and molecular mechanism of this rare female Lesch-Nyhan patient is the total HPRT gene deletion on the maternal X chromosome and the rare specific inactivation of the paternal X chromosome.

SII-4. Fragile X Syndrome: Collaborative Studies of Japanese Patients. Yoshitsugu SUGIO (Div. Med. Genet., Kanagawa Child. Med. Cent., Yokohama)

The fragile X chromosome has attached attention not only to itself but also to X-linked forms of mental retardation. Among Japanese clinicians and cytogeneticists, however, lower attention has been paid. Though a few cytogenetic studies of the fragile X syndrome in various populations have been carried out, the prevalence in Japanese population is not yet available. More studies of the syndrome are needed. Though many patients with the fragile X syndrome are probably in existence, only a few patients were discovered. Most plausible explanation is insufficient understanding of clinical features of the syndrome. Thus, I analyzed clinical features of 31 males with the syndrome in the following eight centers: Ibaraki Colony Asunaro Hosp. (T. Arinami *et al.*), Yamaguchi Univ. (T. Kajii *et al.*), Nagasaki Univ. (N. Niikawa *et al.*), Miyazaki Medical College (S. Ohdo *et al.*), Tokyo Ryouiku Hosp. (Y. Suzuki), Kurume Univ. (T. Matsuishi), Chiba Rehabilitation Center (Kodama), Ryukyu Univ. (T. Ikeda). Most of patients (77%) had IQs below 50. Facial appearance of the adult patients was usually typical for the syndrome, including many characteristic findings such as long face, prominent supraorbital ridge and prominent jaw except for large ears, but in infancy, their facial appearance was almost normal. Careful observation revealed mild mid-face hypoplasia in some cases. Behavioral abnormalities such as hyperactive and autistic movement occurred in only childhood. One-fourth of the patients had epilepsy. Their birthsize was large (mean birthweight at full term 3,431 g). Postnatal overgrowth was also noticed in child age, but final height and weight are usually below the average of normal males. Macrocephaly (+2SD) was observed in one-fourth

of young cases, but the headcircumference of the adults entered into normal range. Mean testicular volume of eight children except one was above the average of normal children, and at least one testis of three children were larger than mean+2SD of normal. Mean testicular volume of eight adult patients was above normal and that of four was below. The frequence and the severity of macroorchidism in Japanese adult patients were both lower than those in Caucasian patients. An increased frequency (40%) of single transverse palmar creases on at least one side was noted (complete type: 25%). The proportion of fra(X) expressing cells in males with high IQ was low. But in low IQ group, the relation between the two was not clear. In this study, there were subtle differences in various clinical features between male patients and normal population. It is, however, doubt that this small differences furnish us with much information to diagnose this syndrome.

A use of excess thymidine in Ham's F-10 was much effective to detect fra(X) in skin fibroblasts, comparing with previous procedures. The fact that proportions of fra(X) positive cells in skin fibroblasts was almost the same as that in peripheral lymphocytes was confirmed in three patients. The result was also same in the case of using Chang medium. In fibroblasts of a mentally retarded female patient, replicating patterns of fra(X) were analyzed using BrdU. The ratio of early to late replicating fra(X) was almost the same as that of her lymphocyte. This method might be usefull for genetic councelling, if the relation between IQ and replicationg patterns of fra(X) would be established. Now, probe F9 and St14 have been used for prenatal diagnosis of the syndrome. Recombination frequencies between the fragile X syndrome and both probes were known to be 10–20% and it was reported that polymorphisms of F9 were not recognized in Japanese population. Thus using only both probes is insufficient. Penetrance of mental retardation in male and female patients is 80–90% and 30–40%, respectively. Even if intragenic probes of this syndrome would be discovered, normal transmitting males and asymptomatic female carriers could not be excluded by this method. If the frequency of fra(X) positive cells in amniocytes would be almost the same as that of skin fibroblasts, chromosomal analysis would be more usefull for prenatal diagnosis than DNA analysis. Since it is suspected that false negative results rarelly occurred in the former, using both analysis is recommended.

SII-5. The Search for Developmental Disorders with Mitochondrial Inheritance.

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Several diseases with non-Mendelian maternal inheritance patterns are described, some of which appear to be associated with abnormal mitochondrial structure or function. Although no human disease has yet been proven to result from the inheritance of an abnormal mitochondrial genome, such defects may be implicated in maternally inherited diseases.

New techniques in molecular genetics should clarify the molecular basis of these maternally inherited developmental disorders.

From the families with maternally inherited mitochondrial myopathy we studied a search for an alteration in nucleotide sequence of the mitochondrial DNA which was obtained from the lymphocytes of patients. Although it was possible to identify a single substitution (C/A) which led to the substitution of Leu/Ile in one of 5 subclones which were obtained from the cytochrome *c* oxidase (CCO) subunit II of the lymphocytes, it was impossible to define the mismatched site by the method of RNA/DNA hybridization using a labelled RNA probe contained the CCO gene which was obtained from normal placenta cells.

A congenital myotonic dystrophy is known to be inherited through an autosomal gene and yet the age of onset and the severity of disease are influenced by non-Mendelian maternal inheritance. We previously proposed that abnormality of bile acid metabolism could affect the fetus, resulting perhaps in delayed development. A possible model for this effect might involve the interaction of the mitochondrial genome with an autosome gene product, such as a DNA-dependent DNA polymerase subunit.