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Review Article

HUMAN GENETICS: PAST, PRESENT, AND FUTURE, WITH SPECIAL REFERENCE TO MAJOR TRENDS IN JAPAN

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The Japan Society of Human Genetics was established in June 1956 [1]. The year 1996 is thus the 40th anniversary of the founding of this Society.

The present article is concerned with recollections, current perspectives, and prospective views of human genetics in the past, present, and future, with special reference to major trends in Japan.

1. Human genetics in the early period

While Mendelism was imported into the Japanese biological societies early in this century and thus led to numerous genetic studies using experimental organisms, relatively few studies had been conducted on human genetics until around 1950 [2, 3].

The eugenic movements had gradually developed from 1930 when the Association of Nation's Hygiene was first organized by a group of medical authorities headed by Hisomu Nagai, the University of Tokyo, who voiced grave concern about the possible "genetic deterioration" of the Japanese nation [3]. Matsunaga (1992) outlined in his article the developmental process of eugenic idea, the National Eugenic Law in the years 1940-1945, and the Eugenic Protection Law in 1948 [3]. Such eugenic movements have since continued to promote the development of human genetics in Japan.

Pioneers of human genetics in Japan. There were pioneers of human

A special lecture commemorating the 40th anniversary of the Japan Society of Human Genetics made in Sapporo City on October 24th, 1996.

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Table 1. Three pioneering studies on human genetics before World War II.

Name of investigators	Year	Title of studies	Reference
Furuhata *et al.*	1925	Proposal of three multiple allele theory for ABO blood groups based upon family studies	[4]
Imai & Moriwaki	1936	Proposal of cytoplasmic inheritance for Leber's optic nerve atrophy	[6]
Komai & Fukuoka	1936	An epidemiological study of twin births	[5]

genetics who published original papers, educational articles, and textbooks relevant to human genetics, while also training a number of junior human geneticists from 1935 to 1955 (Appendix 1). Among the original studies done by these pioneers, three became widely known at the international level (Table 1).

The situations in the first decade following World War II. In the years from 1945 to 1950, when most students started studies of human heredity, genetic studies on experimental organisms were most popular. At that time man was not a favorable subject for studies of this nature for a variety of reasons.

Until around 1955 the students of human genetics, for the most part, focused their attention on the collection of pedigrees in which a given obvious trait appeared, and hence the field of human genetics was regarded as an "uncultivated barren wasteland" in both fields of general genetics and medical biology because of the limitations of its own methodology, and was also often confused with genealogy. Under such circumstances, students of human genetics have thus made great efforts to overcome various obstacles in methodology.

Introductory books to human genetics in the early period. The following textbooks may be listed as those which were invaluable aids for students of human genetics especially in the years from 1950 to 1965.

C. Stern (1949) The Principles of Human Genetics [7]
J.F. Crow (1950) Genetics Notes [8]
J.V. Neel and W.J. Schull (1954) Human Heredity [9]
K. Tanaka (1960) Fundamental Human Genetics [10]

All of these books, except for "Genetics Notes," have since fallen into disuse and have been gradually replaced by more up-to-date versions, but they nevertheless had a great impact on those students. In 1967 a book commemorating "The Mendel Centennial Anniversary in Japan" was published [11].

Establishment of the Japan Society of Human Genetics. In the early 1950s, an increasing number of younger medical investigators became interested in human heredity, but were frustrated because of the lack of mechanisms for communicating with each other. Some of them joined the annual meetings of the Japan Society of General Genetics, but most of them were disappointed since the methodologies employed in experimental genetics were not appropriate for solving their own problems. Studies of human heredity, which preclude the possibility of using crossing experiments, require totally different approaches [2, 3].

In 1955, the need for creating an independent society of human genetics was voiced by a group of younger investigators. This group appealed to a number of colleagues to help establishing the society. Finally, as a result of their efforts, the new society was established [1, 2].

The first meeting of the Society was held at the Keio University Medical School on June 2nd, 1956, and Tanemoto Furuhata was elected to the first president of the Society [1, 2].

International exchanges of knowledge on human genetics at the initial stage. The establishment of the Japan Society of Human Genetics was timely because the First International Conference of Human Genetics was scheduled to take place in Copenhagen in August 1956, while in September 1956 the International Symposium of Genetics was also scheduled to be held in Tokyo and Kyoto, whose programs include a one-day symposium on twin research and human population genetics [3].

Some senior human geneticists including C. Stern, W.C. Boyd, O.v. Verschuer, H. Nachtsheim, R. Turpin, and E. Essenmöller visited Japan to join the symposium. For the majority of human geneticists on the Japanese side it was the first opportunity to have direct discussions with guest speakers. Following the symposia Stern and Verschuer delivered lectures at different universities [3].

The development of human genetics in Japan also owes a lot to J.V. Neel and W.J. Schull of the University of Michigan who have been working in collaboration with Japanese investigators for more than 40 years to study the genetic effects of the atomic bomb in Hiroshima and Nagasaki [12]. They greatly contributed to the development of a number of human geneticists in Japan by demonstrating a leadership in the study of consanguinity in the 1960s and 1970s which will be described later.

In 1956 the first research institute of human genetics was established at the Tokyo Medical and Dental University, and Katumi Tanaka was selected as professor and chairman. This event thereafter led to the establishment of numerous institutions related to human genetics.

Appendix 2 gives a list of the studies and recipients of the Japan Society of Human Genetics Award from 1960 to 1996.

2. Human genetics at the dawn of a new age

In 1955 several breakthroughs were seen in some areas of human genetics, which had been stagnant for a long time, particularly in the fields involving statistical, cytological, and biochemical approaches.

A. Genetics in family and population units

Stochastic and statistical approaches to human heredity had been developed earlier. This stems from a number of sources, but primarily from the fact that human populations are essentially nonexperimental populations and thus not to subject to manipulation by the investigator.

In the 1930s Fisher, Haldane, and Wright developed an elaborate theoretical framework of population genetics, in which they consistently studied various problems in human heredity as well as theoretical aspects of population genetics [13-21].

In the 1940s and 1950s, the field of human genetics involving statistical approaches to family and population data was subdivided into three specified areas: human population genetics, epidemiological genetics, and statistical genetics [9]. Among the studies which have been done by the successors of Fisher, Haldane, and Wright, the lod score method in the linkage test designed by Morton in 1955 is widely used in gene mapping even at the present time [22].

B. Cytological approaches

The number and morphology of human chromosomes could not be identified for 60 years after the first study by Flemming in 1897 [23].

Identification of the number and morphology of human chromosomes. Adapting several advanced techniques to cultured fibroblasts of human embryonic lung tissue specimens, Tjio and Levan found in 1956 that earlier reports had erred in reporting the number of human chromosomes; they found 46 rather than 47 or 48, with the karyotype of females being 46, XX and that of males 46, XY [24].

The first discovery of chromosomal aberration. The development of the method to determine the human karyotype enhanced discoveries of a variety of aberrations in the number and morphology of human chromosomes.

The first example of trisomic chromosomes in man was discovered in 1959 by Lejeune and his colleagues [25]. Their paper is still recognized as one of the classics in human genetics, in that it opened the area of human cytogenetics to intensive investigation. Matsunaga (1993) pointed out the following situation with respect to the rapid spread of chromosome studies: Investigators can obtain direct evidence for mutation through observations of aberrant chromosomes using light microscopy. Such findings soon led to a rapid spread of chromosome studies [26].

Further technical developments. Two other technical developments revolutionized the study of human chromosomes. The first was a report of Caspersson and his associates in 1970 that certain fluorescent derivatives of quinacrine bind differently to different parts of chromosomes [27]. Not long after the fluorescent technique became available, studies in several other laboratories led to the development of alternate techniques for detecting the same banding patterns [28].

The second technical development was the high resolution banding technique [29].

Population cytogenetics. The survey of the incidence of chromosomal aberrations in man was initiated in 1967 by Carr [30]. From 1970 to 1995, a number of attempts were made to estimate the loss of zygotes at the early developmental stages, particularly involving the embryonic, perinatal, and neonatal periods. These data thus led to the approximation of the genetic load due to chromosomal aberrations [31].

C. Biochemical approaches

It has been recognized for many years that a large number of metabolic disorders can be attributed to the congenital deficiency of a specific enzyme, which in turn is due to the presence of a particular abnormal gene. Such conditions were first called "inborn errors of metabolism" in 1902 by Garrod [32].

This was the beginning of biochemical genetics and the idea that genes control the synthesis of enzymes, which in turn are responsible for carrying out specific biochemical process. In 1941 Beadle and Tatum provided experimental evidence for these ideas based on breeding experiments with bread mould *Neurospora crassa* [33].

Inborn errors of metabolism in which the defective enzymes had been previously identified. Phenylketonuria discovered by Fölling in 1934 has been studied extensively [34], and in this inborn error deficiency of phenylalanine 4-hydroxylase was identified in 1953 by Jervis [35]. In 1948 Gibson identified a specific deficiency of the red cell enzyme NADH diaphorase in an autosomal recessive case of hereditary methemoglobinemia, while Takahara and Miyamoto identified a gross deficiency of catalase in acatalasemia in 1949 [36, 37]. Cori and Cori demonstrated in 1952 that a deficiency of glucose-6-phosphatase causes a characteristic form of glycogen storage disease known as von Gierke disease [38].

The first identification of point mutation at the protein level. In 1949 Pauling *et al.* proposed the concept of "molecular disease" based upon a significant difference in mobility in the Tiselius zone electrophoresis between hemoglobins from normal individuals, sickle cell anemia (homozygote), and sickle cell traits (heterozygote) [39]. Using the fingerprinting method of the protein analysis, Ingram showed in 1956 that sickle cell hemoglobin differed from normal hemoglobin in only one amino acid [40]. At a later stage, single amino acid substitution was attributed to single base substitution, which was then the first point mutation identified throughout all organisms.

In the early 1960s population genetics, cytogenetics, and biochemical genetics thus came to be regarded as the three major areas of investigation in human genetics.

3. Biological effects of inbreeding and genetic load

During the past century, in most areas of the world isolates have tended to break up, particularly since World War II. The break-up of isolates results in a change in the level and distribution of consanguineous matings. Sutter and Tabah (1954) were among the first to draw attention to the importance of this process, which they attempted to measure by changes in the demographic variables, *e.g.* endogamy, and consanguineous unions [41]. Nei and Imaizumi (1963) pointed out that the decrease in consanguinity can be partly attributed to a decrease in the mean number of children born and its variance [42]. This theory was proven by a study in a rural community [43, 44].

Consanguinity studies in Japan. The tendency toward endogamy and inbreeding have declined year by year in Japan as well, but it still remains relatively high by world-wide standards [45]. It was therefore a matter of urgency that studies of consanguinity be pursued while there is still time.

For a number of other reasons, Japan was a country suitable for studies on the effects of inbreeding. Particularly, the family registration system, or "*Koseki*," has provided investigators with a set of open records of highly accurate family relationships, and thus are much less subject to errors of recollection than a usual family history [46].

A series of the Japan-US cooperative studies on consanguinity were conducted from 1957 to 1973, under the support of the Genetic Committee of the Science Council of Japan and the University of Michigan [47-49]. The following five urban populations were thus subjected to the studies:

Shizuoka (T. Komai), Hiroshima (W.J. Schull and J.V. Neel),
Nagasaki (W.J. Schull and J.V. Neel), Fukuoka (T. Yanase),
and Hirado (W.J. Schull and J.V. Neel)

The bracketed names show the principal investigators who were responsible for each study [49-54].

In parallel to these studies, more than 30 rural coastal or inland isolated populations located in northern, central, and southern parts of Japan were also investigated [43, 49].

Mortality among the offspring of consanguineous matings. The effects of inbreeding on pre- and postnatal mortalities were studied with the purpose of estimating the amount of hidden genetic load expressed as lethal equivalents per

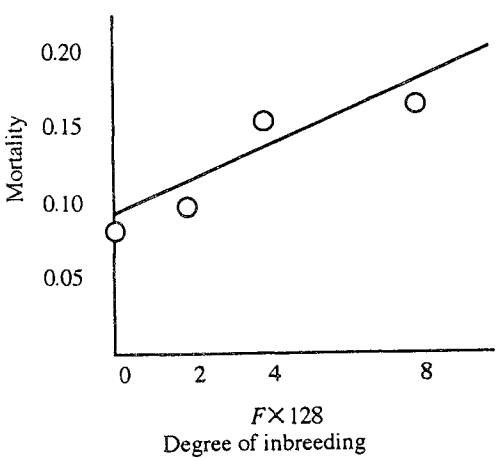

Fig. 1. Regression of early mortality on the degree of inbreeding in an inland rural community.
Yanase 1992 [44].

gamete and zygote. As a result, postnatal mortalities among consanguineous groups were generally higher than those among non-consanguineous groups in all five urban populations. Figure 1 gives the nearly schematic data obtained in a rural community, where a linear regression of the death rates up to six years after birth on the degree of inbreeding (coefficient of in breeding $F \times 128 = 0$, 2, 4, and 8) is shown [44].

Genetic load. The concept of genetic load was first set out by Muller in his special lecture on "our load of mutation" at the annual meeting of the American Society of Human Genetics in 1949 [55], and the theoretical basis of genetic load was thereafter developed largely by Morton, Crow, and Kimura [56-61].

Morton *et al.* (1956) distinguished between the total damage due to disadvantageous mutations present in the human genome and the expressed damage. These were described as lethal equivalents. A lethal equivalent is a group of mutations that, distributed among various individuals, causes one death, on the average, for genetic reasons [14, 56, 57].

In a randomly mating population ($F=0$), the expressed genetic damage—together with the environmental damage—is represented by A. On the other hand, B is a measure for the hidden genetic damage that could manifest itself only with complete homozygosity ($F=1$). The total genetic damage is the sum of B and the genetic component A, and hence, lies between B and $(B+A)$. The linear regression of mortality (m) on degree of inbreeding (F) is thus obtained using by the following formula (Morton-Crow-Muller's model):

$$m = 1 - e^{-(A+BF)} \fallingdotseq A + BF$$

The values of A and B are approximated by the method of maximum likelihood based upon the data on the mortality among the offsprings of consanguineous and non-consanguineous matings ($F=0$, 1/64, 1/32, and 1/16).

The concept of genetic load has been extensively discussed by population geneticists [62-64]. On the other hand, it has also been asserted that of the outcome of consanguineous matings as compared with non-consanguineous matings could contribute to the problem of whether detrimental mutations (mutational load) or balanced polymorphisms due to a heterozygote advantage (segregational load) contribute more to the genetic load of human species [62-64]. Table 2 gives the B/A ratios obtained in the studies of five populations. These lower B/A ratios may be explained by segregation involving a preponderance of

Table 2. B/A ratios in five populations.

Population	A	B	B/A	Ascertainment	Investigator
Shizuoka	0.0647	0.5993	9.3	Followed to 6 years	Tanaka 1973
Fukuoka	0.0682	0.6232	9.1	Followed to 6 years	Yamaguchi *et al.* 1970
Hiroshima	0.0875	0.5317	6.1	Followed to 8 years	Schull & Neel 1965
Nagasaki	0.0986	0.0106	1.1	Followed to 8 years	Schull & Neel 1965
Hirado	0.1157	0.7703	6.7	Largely through age 20	Schull & Neel 1972

References: [50-54].

Table 3. Regression of anthropometric measurements on coefficient of inbreeding (F).

Population	Shizuoka*		Hiroshima**		Nagasaki**	
Range of months of age	76-147		60-132		66-138	
Sex	Male children	Female children	Male children	Female children	Male children	Female children
Stature (mm)	−0.4114	−1.7011	−0.7078	−0.5738	−0.8780	−0.7051
Sitting height (mm)	−0.1304	−1.1279	−0.4647	−0.4154	−0.7288	−0.3167
Chest circumference (mm)	−0.2202	−0.1270	−0.2744	−0.0296	−0.5290	−0.3237
Weight (100 g)	−0.1222	−0.3040	−0.3743	−0.1063	−0.4876	−0.3971
Head length (mm)	−0.1047	−0.1453	−0.1945	−0.0954	−0.0510	−0.1943
Head breadth (mm)	−0.0841	−0.0609	−0.1440	−0.1838	−0.0652	−0.0175
Head circumference (mm)	−0.0054	−0.1750	−0.4648	−0.3009	−0.1763	−0.2057

* Komai & Tanaka 1972, ** Schull & Neel 1965 [49, 51].

balanced genetic systems of various types, *i.e.*, a segregational load.

Physical development of the offspring of consanguineous matings. Table 3 gives the results of a regression analysis of seven anthropometric measurements of school children on the degree of inbreeding (F) in three populations [49, 51]. All variables showed some effect of inbreeding suggesting inbreeding depression upon the physical development.

Human isolates. Human isolated populations may be classified into the following four categories [44]:

(1) Offshoot populations in areas geographically distant from the main population [43].

(2) Communities isolated on account of a particular factor, for example, some 2,300 inhabitants on an island in Nagasaki Prefecture clearly constituted two subgroups: one Buddhist and the other Roman Catholic. Very few marriages were contracted between persons from these two religious groups over the past hundred years [65].

(3) Unique Japanese ethnic communities, such as the Ainu who inhabit the northern most part of Japan [44].

(4) Primitive populations of hunter-gatherers still living under conditions comparable with those during prehistoric times [66].

Isolation obviously affects the breeding structure, migration patterns, founder effect, and particularly genetic distance, its theory and actual applications, in other words factors affecting the evolutionary dynamics of a population. However the biological effects of isolation and its break-up, for example on the differentiation of population subgroups, morbidity, mortality, fertility, *etc.*, should also be considered [44, 67].

4. The development of gene manipulation and the introduction of recombinant DNA technology into medicine

In about 1960 some indications of a second new current in human genetics

were seen after the identification of number and morphology of human chromosomes in 1956. Three discoveries may be pointed out as representative events: the discovery of human leukocyte antigens (HLA) by Dausset in 1958, Philadelphia chromosome by Nowell and Hungerford in 1960, and somatic cell hybridization by Okada in 1962 [68–70]. These works apparently formed the basis of human immunogenetics, cancer genetics, and somatic cell genetics which have since developed with the spread of recombinant DNA technology.

The developmental process of gene manipulation. The first steps toward gene manipulation are thought to have been taken in 1944 when Avery and his associates first demonstrated that genetic information is stored in nucleic acid and not protein as previously believed [71]. In 1952 Hershey and Chase proved that genetic information is transferred by DNA alone [72]. Milestones in DNA technology are listed in Table 4. Emery (1984) stated his impressions in the developmental process of DNA technology as follows [73]:

"After the discovery of the double-helical structure of DNA in 1953, interest

Table 4. Milestones in DNA technology.

1944	Genetic information stored in nucleic acid (DNA) and not protein as previously believed
1953	Double-helical structure of DNA demonstrated
1961	First attempt to break genetic code
1966	Establishment of the complete genetic code
1970	A complete gene synthesized *in vitro* Discovery of the first sequence-specific restriction endonuclease, as well as the enzyme reverse transcriptase
1972	First recombinant DNA molecules generated
1973	Use of plasmid vectors for gene cloning
1975	Southern blot technique for detecting specific DNA sequences
1976	First prenatal diagnosis using a gene-specific probe
1977	Methods for rapid DNA sequencing Discovery of "split genes" and somatostatin synthesized using recombinant DNA
1978	Human genomic library constructed Prenatal diagnosis using linkage with a restriction polymorphism
1979	Insulin synthesized using recombinant DNA
1982	Commercial production of genetically engineered human insulin
1985	DNA fingerprinting introduced Dystrophin gene isolated
1987	Polymerase chain reaction invented
1990	First preimplantation diagnosis carried out
1991	Expanding triplet repeats found in fragile X syndrome and subsequently other diseases
1992	Genethon publish complete genetic map
1993	Genethon complete first human physical map based on yeast artificial chromosomes
1994	Trials of gene therapy in cystic fibrosis begun

Emery & Malcolm 1995 [73].

in moleculer biology grew considerably. The late 1950s and early 1960s were exciting times for geneticists. Unfortunately it gradually became clear that much of what had been learned in molecular biology did not seem to bear much relevance to man, and to medicine in particular. However, interest was dramatically rekindled in the early 1970s with the advent of recombinant DNA technology or, more popularly, genetic engineering. It soon became clear that through the new technology there could be important applications in the prevention and perhaps treatment of genetic disease."

This statement appears to well describe the impressions of most of human geneticists who passed through the era of DNA technology.

Molecular cytogenetics. Identification of chromosomal aberrations using banded karyotypes has been available for some time, but it is often difficult and requires highly trained technicians to identify the exact origin of a derivative chromosome of particularly poor quality preparations [74]. The prevailing situation greatly changed after the introduction of chromosome "paints" for use in fluorescent *in situ* hybridization and microdissection painting.

Langer-Safer *et al.* worked out immunological method for mapping genes on *Drosophila* polytene chromosomes in 1982, and Pinkel *et al.* (1986, 1988) first applied this technique to the analysis of human chromosomes [75-77]. Subsequently, the microdissection of banded human chromosomes was introduced by Ludeck *et al.* (1989) and Senger *et al.* (1990) [78, 79]. Figure 2 shows regional localization of DNA sequences on the corresponding chromosomes which were determined by fluorescent *in situ* hybridization.

The introduction of such new technologies into chromosome studies has thus promoted the development of a new area called "moleculer cytogenetics." This area may cover broad research projects particularly involving the phenomenon of dosage compensation pertinent to X chromosome which was first noted by Lyon in 1961, fragile sites of chromosomes, contiguous gene syndrome, and genomic

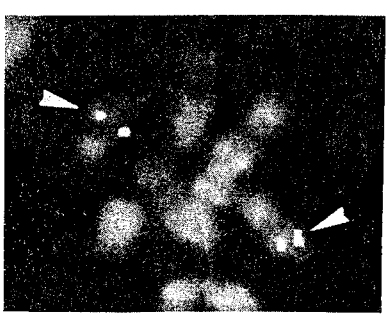

Fig. 2. Fluorescent *in situ* hybridization of probe DNA to chromosome 18 long arm. H. Nakashima with permission (First Department of Medicine, Kyushu University).

imprinting [80-90].

Introduction of DNA technology into medicine. The technology of gene manipulation have introduced many innovations especially into medicine (Table 5). Biogenic peptides and proteins, including more than 40 kinds of cytokine and hormone produced by recombinant DNA technology, have become an invaluable aid in both basic and practical medicine [91-96].

The laboratory examinations undertaken so far were only supplementary means to learn the pathophysiology of diseases, but in the near future these will be potentially valuable tools in the etiological analysis of diseases. The first meeting of "gene diagnosis and therapy" in Japan was held in Kyoto in 1994 [92].

Table 6 gives microbes which can be identified more precisely and rapidly using the combined technology of recombinant DNA and somatic cell genetics. Figure 3 shows a case of *Pneumocystis carinii* pneumonia in which the pathogen was precisely identified by DNA technology.

Table 5. . Introduction of gene manipulation and somatic cell genetics into medicine.

1. Production of biogenic peptides and proteins; cytokines, hormones *etc.*
2. Production of immunological substances against infections; vaccines, monoclonal antibodies, *etc.*
3. Etiological analysis, diagnosis, prophylaxis, and treatment of unifactorial and multifactorial diseases
   (a) Genetical approaches to the pathogenesis of diseases
   (b) Medical diagnosis and gene diagnosis
   Prenatal and postnatal diagnosis, carrier recognition, *etc.*
   (c) Treatment of diseases
   Treatment using biogenic peptides and proteins, gene therapy, *etc.*
4. New laboratory examinations including the gene diagnosis of microbes
5. Phylogenetic classification of microbes
6. Search for DNA polymorphism in the chromosomal and mitochondrial genomes
7. Introduction of recombinant DNA technology into the legal medicine

Table 6. Microbes which can be identified by DNA technology.

Pneumococcal isolates, group A streptococcal isolates, MRSA
Toxic *Clostridium difficile*
Mycobacterial isolates, *Mycobacterium tuberculosis*, pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Sallmonella* strains, *Legionella pneumophilia*
Borrelia burgdorferi, *Treponema pallidum*
Rickettsia rickettsi
Chlamydia species
Candida species
Human papilloma virus, hepatitis C virus, human immunodeficiency virus, cytomegalovirus, herpes simplex virus
Pneumocystis carinii

Miyaji 1996 [93].

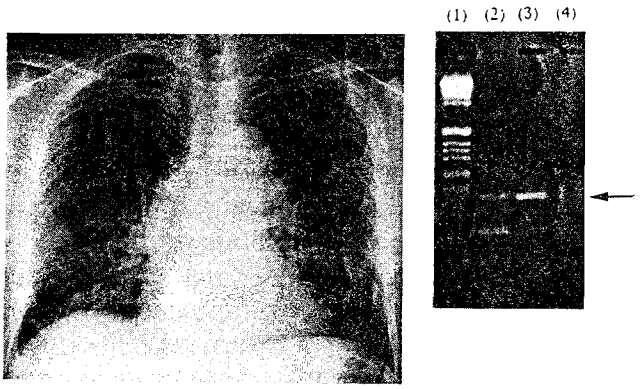

Fig. 3. DNA diagnosis of *Pneumocystis carinii* pneumonia.
(1) DNA size marker, (2) positive control, (3) sample from the patient, and (4) negative control. R. Kuboi with permission (First Department of Medicine, Kyushu University).

Table 7. Subjects on genetics addressed at the annual meetings of the Japan Society of Internal Medicine (1990-1996).

Year	Number of subjects	Percentage of subjects related to genetics (%)	Percentage of subjects studied by means of gene manipulations (%)
1990	554	9.2	5.6
1993	757	18.4	7.1
1996	756	25.4	11.2

J Jpn Soc Intern Med, Vol. 79, 82, and 85 (special issues) [96].

Judging from these trends, the re-classification of microbes including virus, bacteria, rickettsia, chlamydia, mycoplasma, spirochete, treponema, and protozoan may be an important issue in the future, and the field of microbiology may change the entire aspects of knowledge and technology, if microbes can be adequately classified into families, species, and subspecies based upon moleculer phylogenetic trees which are usually made by a comparison of the base sequence in ribosomal RNA between microbes [92-95].

The method of polymerase chain reaction (PCR) has greatly contributed in these developmental processes [91].

Table 7 gives the number of subjects relevant to genetics which were presented in the past six years at the annual meetings of the Japan Society of Internal Medicine, the greatest society of clinical medicine in Japan. In 1996 the number of subjects on genetics has increased by three times compared to that in 1990.

5. Human hemoglobins and hemoglobinopathies

Human hemoglobins have contributed remarkably to the overall understanding of the basic genetic principles and molecular basis of morbidity since the first

such study by Ingram in 1956. Further studies of importance were the establishment and elucidation of the full amino acid sequence of the hemoglobin chains by Braunitzer *et al.* in 1961, and of the three-dimensional structure of mammalian heme proteins by Kendrew *et al.* in 1960 and Perutz in 1976 [97-99].

There are a number of reasons for this key role of hemoglobin, including the fact that it occurs in relatively accessible cells, in a relatively pure form, and in relatively large amounts [74]. In addition, the introduction of DNA technology into the study of hemoglobins has also resulted in the following progress:

The reticulocyte contains only globin mRNA, and therefore if this mRNA is exposed to reverse transcriptase it is possible to make a globin cDNA copy. The isolation of the hemoglobin mRNA has led to new insights into the gene structure and function. Restriction enzymes have been utilized for a refined analysis of the structure of hemoglobin genes [73, 100, 101].

Multigene family encoding the globin genes. The multigene family encodes the human globins, which will be discussed in Section 7. The α-like globin genes are located as linked clusters on chromosome 16, in order of 5′-ζ-$\psi\zeta$-$\psi\alpha$-α2-α1-θ-3′, and the β-like globin genes on chromosome 11, in order of 5′-ε-$^{G}\gamma$-$^{A}\gamma$-$\psi\beta$-δ-β-3′. The loci designated $\psi\alpha$, $\psi\beta$, and $\psi\zeta$ are called pseudogenes which have a sequence homology with the α, β, and ζ genes but have mutations that prevent their expression [102, 103]. It seems likely that they are evolutionary remnants of once active globin genes [104, 105].

Hemoglobin and the genetic code. Studies of human hemoglobins were thus able to quickly assess the adaptability of the genetic code. In 1969 Lehmann and Carrel examined the 95 amino acid substitutions known for human hemoglobins and first assessed the adaptability of the genetic code [106].

The phenomenon of split genes and RNA splicing in the globin genes. Shortly after the discovery of RNA splicing and split genes in an adenovirus by Sharp [107], Jeffreys and Flavell (1977) found in the β-globin of the rabbit that during transcription the precursor RNA derived from introns is excised and precursor RNA from non-contiguous exons is spliced together to form functional mRNA [108]. The same phenomenon was also ascertained in the human globin genes [109].

Almost all the genes in eukaryotes are split by introns, although in man there are few exceptions such as the genes for histones, interferons, mitochondrial genome, and the sex-determining gene, *SRY*. The function of introns is not clear, but mutations within the introns may seriously affect RNA splicing and thereby interfere with correct gene synthesis [73]. There are a number of examples of such mutations in hemoglobinopathies (Table 9).

Discovery of restriction fragment length polymorphism. Jeffreys (1977) discovered in the region of β-like globins in man that variations occur as frequently as once in every hundred base pairs [109, 110]. Since these variations are relatively frequent in the general population and are recognized by differences in

the restriction fragment lengths, they were referred to as restriction fragment length polymorphism (RFLP). Such polymorphism has greatly contributed to advances in gene mapping.

Locus control region. Experiments involving the transfer of increasingly long segments of DNA into mouse embryos (transgenic mice) have revealed the need for a stretch of DNA, called a locus control region (LCR), upstream from the structural genes, in order to regulate the expression of the gene cluster correctly [111]. A type of $\gamma\beta$-thalassemia was also found to result from a deletion of this LCR [73, 114].

The first attempt of prenatal diagnosis. Kan *et al.* (1976) demonstrated by means of DNA technology that if the fetus was affected by α-thalassemia, a disorder in which globin genes are deficient, then there was a reduced hybridization with an appropriate globin cDNA probe prepared from reticulocyte mRNA [112]. This thus represented a monumentary step in the field of prenatal diagnosis [73].

Molecular pathology of hemoglobinopathies and new genetics. A research area of approaches at the molecular level to the pathogenesis of unifactorial and multifactorial diseases was called "molecular pathology" by Perutz and Lehmann in 1968 [113], and the term "new genetics" was first used by Comings in 1979 for a novel approach in using DNA analysis for mapping the human genome [104].

By far the majority of visible mutations involve single base substitutions. In addition to these, various kinds of other mutations have also been identified including deletion, insertion, duplication in the base sequence, premature termination, alteration of stop codon leading to elongation of globin chains, frame shift,

Table 8. Structural variants of hemoglobin with specific morbid conditions discovered in Japan.

Variant	Reference	Variant	Reference
Sickle cell trait		*Hemoglobins with high oxygen affinity*	
Hb S	Shibata *et al.* 1980	Hb Chesapeake	Harano *et al.* 1983
Unstable hemoglobins		＊Hb Hirose	Yamaoka 1971
Hb Bristol	Shibata *et al.* 1985		Sasaki *et al.* 1978
Hb Genova	Shibata *et al.* 1980	＊Hb Hiroshima	Hamilton *et al.* 1969
Hb Hammersmith	Miyaji *et al.* 1975	Hb Province	Pc
Hb Koln	Shibata *et al.* 1961	Hb Rahere	Sugihara *et al.* 1985
＊Hb Mizuho	Ohba *et al.* 1977	Hb San Diego	Pc
＊Hb Showa-Yakushiji	Kobayashi *et al.* 1987	*Hb M diseases*	
	Naritomi *et al.* 1988	Hb M Boston	Hayashi *et al.* 1964
＊Hb Tochigi	Shibata *et al.* 1970	Hb M Hyde Park	Shibata *et al.* 1963
Hemoglobins with low oxygen affinity		＊Hb M Iwate	Miyaji *et al.* 1963
Hb Kansas	Pc	Hb M Saskatoon	Shibata *et al.* 1961
＊Hb Yoshizuka	Imamura *et al.* 1969		

＊ Variants first discovered in Japanese. Pc: Personal communication.
Compiled by T. Imamura (National Institute of Genetics, Mishima).

Table 9. Mutations in the thalassemias and allied conditions.

1. *Deletion*
   - Total deletion of the gene
   - Partial deletion of the gene
2. *Mutations affecting translation*
   - Nonsense mutations
   - Frameshift mutations
   - Initiation codon mutations
3. *Mutations affecting transcription*
   - Mutations in the CAC box
   - Mutations in the ATA box
   - Mutations in the GATA motif
   - Mutations in the cap site
4. *Mutations affecting RNA processing*
   - Mutations involving breakage at the 3′ side of pre-RNA
   - Mutations affecting splicing
     - Mutations inactivating donor or acceptor sites
     - Mutations activating cryptic splice sites or creating new splice sites within exon or intron
5. *Mutations affecting the post-translational process*
   - Mutations inducing degradation of globin chains

Compiled by Y. Fukumaki (Institute of Genetic Information, Kyushu University).

unequal crossover, and fusion of genes, *etc.* These genomic changes resulted in a number of functional alterations and morbid conditions: modification of allosteric properties (hemoglobin variants with increased or reduced oxygen binding leading to polycythemia or cyanosis), reduced stability of the hemoglobin molecule (hemoglobin variants leading to hemolytic anemia).

The structural variants with specific morbid conditions discovered by Japanese investigators are listed in Table 8.

Table 9 gives a greater variety of mutations identified in the thalassemias and allied conditions which are characterized by defects in the sythesis of globin chains. Today, most of the conditions can be explained by point mutations or gene deletions that modify the output from a mutant locus [114–120].

There are two examples of particular interests among these variants: Hb Constant Spring and Hb Lepore. The former variant results from a mutation which eliminates the normal stop codon in α-globin gene with the result that the α-chain is unusually long and unstable, thus showing the clinical picture of α-thalassemia [121]. Hb Lepore, which was first discovered by Gerald and Diamond in 1958, showed the symptoms of β-thalassemia due to an unequal crossover between the β- and δ-globin loci [122]. Thereafter two examples of the opposite crossover products were discovered and called anti-Lepore hemoglobin: Hb P Congo and Hb Miyada [123, 124]. Since individuals of anti-Lepore hemoglobins possess a full set of normal β-globin genes, in addition to the β-δ fusion gene, β-chain synthesis is thus considered to be normal.

Table 10. List of G6PD variants found in Japanese.

Class*	Variant	Properties**	Nucleotide change	Amino acid change	References and remarks
I	Tokyo	1S:NNN:NNN:LN	1246 G→A	416 Glu→Lys	Hirono *et al.* 1992
I	Wakayama	1S:NNL:N-N:LN	n.d.***	n.d.	Miwa *et al.* 1978
I	Kurume	1S:NNL:N-N:LB	n.d.	n.d.	Miwa *et al.* 1978
I	Fukushima	1S:NNL:N-L:LN	1246 G→A	416 Glu→Lys	Miwa *et al.* 1978
I	Yamaguchi	1S:NHN:N-H:LS	1160 G→A	387 Arg→His	Miwa *et al.* 1978
I	Iwate	1S:NHL:NNN:LN	1160 G→A	387 Arg→His	Kanno *et al.* 1988
I	Asahikawa	1S:NHL:HNH:LB	695 G→A	232 Cys→Tyr	Beutler *et al.* 1996
I	Niigata	1S:NHL:HHL:LA	1160 G→A	387 Arg→His	Chiba *et al.* 1989
I	Sendagi	1S:LHN:H-H:LN	n.d.	n.d.	Morisaki *et al.* 1983
I	Kobe	1S:HNN:NHH:LA	1318 C→T	440 Leu→Phe	Hirono *et al.* 1994
I	Tsukui	1S:HNN:N-L:LN	3 base→del	188 Ser→del	Hirono *et al.* 1995
I	Gifu	1N:NNN:NNH:LN	n.d.	n.d.	Fujii *et al.* 1984
I	Akita	1N:NNN:N-N:LN	n.d.	n.d.	Miwa *et al.* 1978
I	Kanazawa	1N:NNN:N-N:LB	n.d.	n.d.	Kitao *et al.* 1982
I	Ogikubo	1N:NNN:H-N:LN	n.d.	n.d.	Miwa *et al.* 1978
I	Yokohama	1N:NNL:N-N:LN	n.d.	n.d.	Miwa *et al.* 1978
I	Morioka	1N:NNH:NNH:LN	1339 G→A	447 Gly→Arg	Endo *et al.* 1985
I	Sapporo	1N:NNH:HHH:LN	n.d.	n.d.	Fujii *et al.* 1981
I	Tokushima	1N:NHN:NNN:LN	n.d.	n.d.	Miwa *et al.* 1976
I	Kyoto	1N:HH-:---:LA	n.d.	n.d.	Kojima 1972
I	Nagano	1F:LHL:NNN:LN	n.d.	n.d.	Takahashi *et al.* 1982
I	Fukuoka	1F:HNN:N-H:LB	n.d.	n.d.	Fujii *et al.* 1984
I	Heian	1F:HH-:H-H:-B	n.d.	n.d.	Nakai & Yoshida 1974
I	Shinagawa	1-:HH-:NNN:LN	1229 G→A	410 Gly→Asp	Hirono *et al.* 1994
I	Shinshu	1-:HH-:NNL:NN	527 A→G	176 Asp→Gly	Hirono *et al.* 1994
I	Nara	1-:—:—:L-	953-976→del	319-326→del	Hirono *et al.* 1993
2	Onoda	2S:LN-:H-H:LB	n.d.	n.d.	Unpublished, cited in Beutler & Yoshida 1988
2	Ogori	2N:NNN:NNN:NN	n.d.	n.d.	Miwa *et al.* 1977
2	Mediterranean-like	2N:LL-:HHH:LB	n.d.	n.d.	Miwa *et al.* 1977
2	Urayasu	2F:NNL:NNN:NN	281-283→del	95 Lys→del	Hirono *et al.* 1995
3	Musashino	3S:NLN:NHH:NN	185 C→T	62 Pro→Phe	Kuwakawa *et al.* 1987
3	Kimiube	3N:NNN:N-N:NN	1387 C→T	463 Arg→Cys	Nakatsuji & Miwa 1979

Table 10. (continued)

Class*	Variant	Properties**	Nucleotide change	Amino acid change	References and remarks
3	Hofu	3N:LNN:NNH:NN	n.d.	n.d.	Miwa *et al.* 1977
3	Konan	3F:NNN:N-N:NN	241 C→T	81 Arg→Cys	Hirono *et al.* 1993
4	B	4N:NNN:NNN:NN			Wild type
4	Kiwa	4F:NNN:N-N:NN	n.d.	n.d.	Nakatsuji & Miwa 1979

*1: associated with nonspherocytic hemolytic anemia; 2: severely deficient, less than 10% residual activity; 3: moderately deficient, 10–60% residual activity; 4: normal activity: 60–150%; 5: increased activity. **(class, electrophoresis: Km-G6P, Km-NADP, Ki-NADPH: 2deoxy-G6P, gal-6P, deamino-NADP: thermostability, pH-optimum); N: normal, H: high, L: low, B: biphasic, A: acidic. ***n.d.: not determined.

Compiled by A. Hirono (Okinaka Memorial Institute for Medical Research, Tokyo).

In addition to models of the mutation shown in the Tables 8 and 9, there are also a number of unifactorial diseases due to new classes of pathogenic mutations which have been identified in the relatively recent past. These new classes of pathogenic mutations will be presented in Section 9.

6. Glucose-6-phosphate dehydrogenase variants and nonspherocytic hemolytic anemia

The discovery of X-linked variations of glucose-6-phosphate dehydrogenase (G6PD) followed the finding that a significant proportion of American Blacks develops acute hemolysis when they receive the synthetic antimalarial drug, primaquine [125]. It was shown that the hemolytic response to the drug was due to an intrinsic red cell abnormality, the red cell glutathione level was markedly decreased in primaquine sensitive individuals, and these were caused by a specific deficiency of the enzyme G6PD [126–128]. Since that time, a variety of variants of the enzyme G6PD have been identified biochemically.

In the early 1960s, the four alleles, Gd^B, Gd^A, Gd^{A-}, and $Gd^{Mediterranean}$ appeared to determine four structually distinct forms of the G6PD enzyme protein [130, 131]. The B enzyme was regarded as the wild type, because it was the most common and occurred in all populations. The others possibly differed from it simply by single amino acid substitutions. Evidence on this point was demonstrated by Yoshida (1967): a particular asparagine residue presents in the B enzyme is replaced by an aspartic acid residue in the A enzyme [132]. In 1986, amino acid sequence of human G6PD was deduced from the cDNA sequence [133, 134]. The tertiary structure of G6PD from *Leuconostoc mesenteroides* has recently been determined [135], and a model of the tertiary structure of the human enzyme has also been subsequently deduced [136].

Glucose-6-phosphate dehydrogenase variants. It became apparent almost immediately after the discovery of G6PD deficiency that the investigator was

dealing not with a single mutation, but with a variety of genetic changes [129]. In 1967, a WHO study group recommended standardized methods of biochemical analysis, which have been followed by most investigators [137]. As a result, more than 400 different variants have so far been identified. On the other hand, the DNA mutations causing G6PD deficiencies have been analyzed since 1987 [138], and nearly 100 molecular variants have been reported so far [139]. Table 10 summarizes the variants found in Japanese, most of which were identified by Miwa and his associates [140]. Luzzatto and Metha (1995) commented as follows:

Molecular analyses have confirmed that the basis for G6PD deficiency is widely heterogeneous. In some cases, variants that had been assigned different names turned out to be identical; conversely, however, some variants that had been thought to be homogeneous have turned out to be heterogeneous on the moleculer level. Different mutants, each one having a polymorphic frequency, underlie G6PD deficiency observed in various parts of the world where this abnormality is prevalent. Genetic heterogeneity also explains, to a large extent, the diversity of clinical manifestations. Different mutations are responsible for the less common patients who have chronic hemolytic anemia and for the frequent patients who are only at risk of developing episodic hemolysis [129].

7. Population genetics

As a branch of biology, the origin of population genetics research goes back to Darwin's theory of evolution and Mendel's theory of inheritance which were synthesized with the biometrical methods developed by Pearson, Galton, and Weldon. It is well known that this synthesis was accompanied by the works of the great triumvirate, Fisher, Haldane, and Wright [13]. The present section focuses on four topics of population genetics relevant to human genetics.

Multigene families. Certain traits in man are controlled by several genes with related functions which occur either on the same chromosome, on two different chromosomes, or on several different chromosomes. For example, (1) the α interferon genes on chromosome 9, and the major histocompatibility gene complex on chromosome 6, (2) α globin and β globin are related to the gene loci of hemoglobin on chromosome 16 and 11 respectively (p. 277), and (3) argininosuccinate synthetase which is dispersed over at least eight autosomes and both sex chromosomes [73].

For a particular multigene family, different gene members within the same species show a much higher homology in amino acid and base sequences than those between different species. This mode of evolution is called coincidental evolution or concerted evolution which was first studied by Smith in 1974 [141]. Ohta (1982, 1983, 1991) showed through statistical analyses of multigene families that concerted evolution can quantitatively be explained by repeated gene duplication, unequal crossover, and sometimes gene conversion [142-144].

Genetic distance. In 1953 Sanghvi proposed the concept of genetic distance

in his study of the genetic differentiation of human populations, and the theoretical and practical approaches to this concept have further been developed by Cavalli-Sforza and Edwards (1967) [145, 146].

Harris and his associates (1966) made it possible to estimate the frequencies of alleles at a particular locus in human populations in a direct fashion with the advent of electrophoretic methods capable of being applied to a wide range of different enzymes and protein components [147]. This technical approach facilitated studies of genetic distance.

Among three models measuring genetic distance developed by Nei (1972), the standard genetic distance has widely been used particularly in the field of human genetics [148, 149]. Table 11 shows the genetic distance (D) between Caucasoids, Negroids, and Mongoloids which was estimated using Nei's formula. The genetic distance between Negroids and Mongoloids is slightly higher than that between Caucasoids and Negroids. On the other hand, the distance between Caucasoids and Negroids is the least among the three pairs of groups [149].

The neutral theory of moleculer evolution. The neutral theory of moleculer evolution was first proposed by Kimura in 1968. Using Haldane's theory of the cost of selection, Kimura estimated that the genetic load would be too large to maintain the entire human population if selection operated on the whole genome and he thus came to the conclusion that most mutations occurring in the genome would have to be selectively neutral or nearly neutral [150].

In 1969, King and Jukes in support of the neutral theory, independently proposed that non-Darwinian evolution is prevalent at the molecular level, showing that for protein-coding regions, nucleotide substitutions at the third codon positions take place more frequently than those at the second and first codon positions [151]. There have also been critics of the neutral theory and discussions on the neutral theory have thus developed into the so-called neutralist-selectionist controversy.

In 1971, Kimura and Ohta demonstrated clearly that moleculer evolution and genetic polymorphism can be explained in a unified way by the neutral theory, if genetic polymorphisms are regarded as an intermediate state during the stage in which a new mutation can proceed toward fixation in a population [152]. In 1976, Nei and his colleagues found support for the neutral theory in the results of

Table 11. Minimum, standard, and maximum estimates of number of net codon differences between Caucasoid, Negroid, and Mongoloid populations.
A comparison using 35 common protein loci.

	Caucasoid/Negroid	Caucasoid/Mongoloid	Negroid/Mongoloid
Minimum	0.014	0.010	0.017
Standard	0.017	0.011	0.019
Maximum	0.021	0.012	0.026

Nei & Roychoudhury 1974 [149].

statistical analyses of a large amount of data on protein polymorphisms in a number of organisms including man [153]. In addition, Ohta proposed the slightly deleterious mutation theory as a modified extension of the neutral theory to successfully explain the observation that rare alleles were significantly in excess of that expected under the assumption of the neutral theory. She contended that most of the alleles which proceed to fixation are slightly deleterious rather than completely neutral [154].

Recent advances in DNA research have provided geneticists with substantial evidence supporting the neutral theory. First of all, Miyata *et al.* (1980) demonstrated that synonymous substitutions take place at a much higher rate than nonsynonymous substitutions. This indicates that a mutation that does not lead to an amino acid substitution is a neutral mutation at least at the protein level [155]. More crucial support for the neutral theory was provided by Li *et al.* and Miyata and Yasunaga in 1981. They showed that the rate of nucleotide substitutions in pseudogenes were the highest among the genes examined. Since pseudogenes are replicas of active genes but do not yield a recognizable gene product, the evolution of pseudogenes may thus be regarded as a paradigm of neutral evolution [156, 157]. Furthermore, the neutral theory is also supported by observations that the extremely high rates of nucleotide substitution in RNA viruses cannot be explained by strong positive selection but rather by an extremely high mutation rate [158].

The neutral theory has been accepted by many investigators, but further studies are still needed to elucidate how many loci evolve in a manner that departs from the neutral theory [159, 160].

Studies to determine spontaneous and induced mutation rates in human germ cells. After extensive epidemiological studies of the children of atomic bomb survivors which have yielded no statistically significant increases in the genetic effects compared to a control population [161], beginning in 1976, an approximately 10 year investigation to detect germ cell mutations at the protein level was carried out. Two groups of children, that is, 11,364 children of the exposed parents in which the combined parental gonadal dose of each child was 0.01 Sievert (Sv) or more (exposed group), and 12,297 control children in which the combined parental dose was less than 0.01 Sv, were examined for rare (allele frequency is less than 0.01) electrophoretic variants of 30 blood proteins; 10,000 of these children were also screened for enzyme-deficiency (66% or less of the mean normal enzyme activity) variants of nine erythrocyte enzymes. In addition to approximately 1,200 inherited variants, three mutants in the exposed group, and four in the control group were detected. No statistically significant difference was observed between the mutation rate of the exposed group and that of the control group [162-165]. It is possible, however, that the number of loci screened for mutations might not have been large enough to allow detection of any radiation-induced mitations.

Recently, the feasibility of detecting radiation-induced mutations at the DNA level has been explored. To serve as a source of cells for future screening, cell lines have been established from the peripheral B lymphocytes of 800 families composed of father-mother-child trios from the exposed and the control groups.

For detection of deletion/insertion/rearrangement (D/I/R) types of mutations, which are believed to predominate among radiation-induced mutations, repetitive sequences such as minisatellites and microsatellites are being examined in a pilot study. A subsample of the 800 families, composed of 50 exposed families and 50 control families are being used. Preliminary results show that 58 mutations were detected in a total of 124 children. These germ cell mutations, however, showed no evidence of being induced by radiation [166-168]. Two dimensional gel electrophoresis of DNA, which has been shown to be suitable for screening for D/I/R mutations in single copy sequences [169], will also be employed in the pilot study. With these techniques, frequencies of spontaneous and induced mutations at various parts of DNA in human germ cells will be obtained at the DNA level.

8. Entities of hereditary disease, inborn error of metabolism, and congenital abnormality established by Japanese investigators

Tables 12 and 13 show the entities of hereditary disease, inborn error of metabolism, and congenital abnormality which have been established by careful and sharp-sighted observations of Japanese investigators in the relatively recent

Table 12. Inborn errors of metabolism discovered by Japanese investigators (1949-1994).

Disease entity	Investigator
Acatalasemia	Takahara & Miyamoto 1949
Pyruvate kinase deficiency	Tanaka KR *et al.* 1962
Formiminotransferase deficiency	Arakawa *et al.* 1965
Glycogen disease type VII	Tarui *et al.* 1965
Isovaleric aciduria	Tanaka K *et al.* 1966
GM1-gangliosidosis	Okada & O'Brien 1968
GM2-gangliosidosis	Okada & O'Brien 1969
Nonketotic hyperglycinemia type I	Tada *et al.* 1969
Globoid cell leukodystrophy	Suzuki & Suzuki 1970
Hereditary progressive dystonia	Segawa *et al.* 1976 Ichinose *et al.* 1994
Glycogen disease type IB	Narisawa *et al.* 1978
Nonketotic hyperglycinemia type III	Hayasaka *et al.* 1983
Tyrosinemia type III	Endo *et al.* 1983
Methylglutaconic aciduria	Narisawa *et al.* 1986
Cerebrohepatorenal syndrome	Shimozawa *et al.* 1992
Hereditary motor and sensory neuropathy type I	Hayasaka *et al.* 1993
Dentatorubral-pallidoluysian atrophy	Koide *et al.* 1994 Nagafuchi *et al.* 1994
Machado-Joseph disease	Kawaguchi *et al.* 1994

past. Among these, four unique disorders are outlined here.

Muscle phosphofructokinase (PFK) deficiency. Muscle PFK deficiency described first by Tarui and his associates in 1965 was classified as type VII glycogenesis, and no controversy has hitherto occurred concerning the eponym [170]. This is a clinical entity first demonstrated among the enzyme defects in muscle glycolysis, if the word of "glycolysis" is used separately from "glycogenolysis." This disease entity has also been added to the group of enzymopenic hemolytic diseases. Among eight clinical entities of hepatic and muscle glycogenosis, in addition, the causal genetic lesion was first demonstrated by Tarui and his associates in 1990 by an analysis of muscle PFK deficiency.

It is unparalleled that this individual research group was able to not only establish a novel clinical entity but also to demonstrate the causal defective enzyme, the thorough pathophysiology, to succeed in cDNA cloning of the normal human enzyme, and to identify gene structure for the normal enzyme and the responsible several genetic defect [171].

Nonketotic hyperglycinemia (NKH). NKH is an autosomal recessive disorder characterized by abnormally high concentrations of glycine in plasma and cerebrospinal fluid, causing severe brain damage in the early stages of life. In 1969 Tada and his associates demonstrated that the primary defect is in the glycine cleavage system, which consists of four protein components: P, H, T, and L. This research group revealed that the majority of NKH patients had a specific defect in P-protein (type I), some patients had a specific defect in T-protein (type II), while one patient had a specific defect in H-protein [172, 173].

The cDNA encoding human P- and T-protein respectively, and the molecular lesions at the DNA level were elucidated in the patients with NKH. Based upon these results, Tada pointed out that the glycine cleavage system is a physiologically

Table 13. Hereditary diseases and congenital abnormalities discovered by Japanese investigators (1960-1988).

Disease entity	Investigator
* Fukuyama congenital muscular dystrophy	Fukuyama *et al.* 1960
Cerebro-oculo-hepato-renal syndrome	Arima *et al.* 1971
* Conotruncal anomay face syndrome	Takao *et al.* 1980
Kabuki make-up syndrome	Kuroki *et al.* 1981
	Niikawa *et al.* 1981
* EEM syndrome	Ohdo *et al.* 1983
* Tricho-rhino-phalangeal syndrome type III	Sugio & Kajii 1984
Ohdo blepharophimosis syndrome	Ohdo *et al.* 1986
TAEDLD abnormalities	Ohdo *et al.* 1987
Sonoda syndrome	Sonoda *et al.* 1988
Cryptomicrotia-brachydactyly syndrome	Tonoki *et al.* 1988

* Modes of inheritance were determined. EEM syndrome: Ectodermal dysplasia, ectrodactyly, and macular dystrophy, TAEDLD abnormalities: Tetra-amelia with ectodermal dysplasia and lacrimal duct abnormalities.

major pathway of glycine catabolism in man and other mammals, and this system thus plays an important role in regulating the concentrations of glycine in the brain which is neurotoxic when excessively produced [173].

Fukuyama congenital muscular dystrophy (FCMD). Fukuyama congenital muscular dystrophy (cerebromuscular dystrophy; Fukuyama type) is an autosomal recessive disorder which was first described by Fukuyama and his colleagues in 1960 [174]. This disease entity is clinically characterized as follows: (1) an early onset in infancy, usually before the age of 9 months, (2) muscle hypotonia and weakness in infancy, (3) wasting of the muscles and contracture of the joints gradually developing towards the later stages, particularly involving the proximal muscles, (4) myopathic facies, (5) pseudohypertrophy of calf muscles and febrile or non-febrile convulsions (in approximately 50% of the cases), (6) mental retardation with speech disturbance, *etc* [174].

All available examinations of skeletal muscles revealed changes which were essentially identical with those in Duchenne type of muscular dystrophy. Neuroimaging technologies and autopsies of more than 50 cases undertaken in Japan also results in new insights onto malformation in the brain including a remarkable malformation of cobblestone lissencephaly (polymicrogyria and pachygyria), brain stem dysplasia, *etc.*

The gene responsible for the development of FCMD is located on chromosome 9 (9q31-33) [175].

Kabuki make-up syndrome. This congenital abnormality is a causally unknown multiple congenital anomaly/mental retardation syndrome which was independently described by Kuroki *et al.* and Niikawa *et al.* in 1981 [176, 177].

Most affected individuals had five cardinal manifestations: (1) a peculiar facial expression characterized by an eversion of the lower lateral eyelids, arched eyebrows with sparse or dispersed lateral one-third, depressed nasal tip, and prominent ears, (2) skeletal anomalies including brachydactyly V and spinal deformity with or without sagittal cleft vertebrae, (3) mild to moderate mental retardation, (4) postnatal growth disturbance, and (5) dermatoglyphic abnormalities. Various complications were observed including congenital defects in the cardiovascular system, early breast development in infantile females, cleft lip and/or palate, recurrent otitis media in infancy, renal/urinary tract anomalies, seizures with abnormal EEG findings.

Five familial cases have been accumulated so far. In addition, an autosomal dominant mode of inheritance with variable expressivities has also been proposed at the present time [176, 177].

9. New classes of pathogenic mutation

In the 1980s and 1990s a number of new classes of pathogenic mutation have successively been identified, which differed from the hitherto known mutational changes in the structural genes on chromosomes (Table 14). The following

Table 14. New classes of pathogenic mutation.

Somatic mutation
Change in mitochondrial genome
Mismatch DNA repair
Deletion or duplication of contiguous genes
Uniparental disomy involving genomic imprinting
Unstable number of trinucleotide repeats
Increased chromosomal instability
Mutation affecting translation
Mutation affecting transcription
Mutation affecting RNA processing
Mutation affecting post-translational process

unifactorial diseases may be pointed out as such examples: paroxysmal nocturnal hemoglobinuria due to somatic mutation, Kearns-Sayre syndrome due to a change in mitochondrial genome, a type of *Xeroderma pigmentosum* due to mismatch repair as copy editing for errors that have occurred during replication, contiguous gene syndrome due to deletion or duplication of contiguous genes, Prader-Willi syndrome due to uniparental disomy (genomic imprinting), neurodegenerative diseases and fragile X syndrome due to unstable number of trinucleotide repeats, Bloom syndrome due to increased chromosomal instability, and thalassemias and allied conditions due to mutations affecting translation, transcription, RNA processing, and post-translational process.

Among these deseases, two examples are herein outlined.

Paroxysmal nocturnal hemoglobinuria (PNH). In 1964 Ross and Rosenbaum suggested that PNH might be a disease due to somatic mutation because of the presence of two red cell populations [179].

Many eukaryotic proteins bind to the cell membrane by a glycosyl phosphatidylinositol (GPI) anchor. Kinoshita and his associates demonstrated that the biosynthesis of GPI anchor is deficient in the abnormal blood cell populations of this disease. A deficiency in the surface expression of GPI-anchored complement inhibitors leads to complement-mediated hemolysis. The *PIGA* gene responsible for the biosynthesis of the GPI anchor lies on chromosome X, and the mutant *PIGA* gene accounts for the expression of X-linked recessive phenotype of somatic mutation. The somatic mutation must occur in the hematopoietic stem cell because the abnormal cell population appears in all blood cell lineages. These mutations tend to vary among the affected individuals and are usually small, involving one or two bases [180, 181].

Dentatorubral and pallidoluysian atrophy (DRPLA). This neurodegenerative disease entity, DRPLA, is an autosomal dominant disease which was first described by Koide *et al.* and Nagafuchi *et al.* independently in 1994 (Table 12). DRPLA is characterized by the combined degeneration of the dentatorubral and pallidoluysian systems, showing a combination of symptoms including cerebellar

ataxia, myoclonic epilepsy, choreoathetosis, and dementia. The affected individuals possess an expanded CAG trinucleotide repeat in a gene on the short arm of chromosome 12. The size of repeats varied from 7 to 23 in normal individuals, but in affected individuals one allele was expanded to 49-57 repeats or occasionally even more. Expansion was usually associated with paternal transmission and only occasionally with maternal transmission. The size of the repeats showed a close correlation with the age of onset and severity of the disease. The expansion of the triplet repeats accounted for a number of clinical characteristics such as the phenomenon of anticipation and a wide variety of clinical manifestations of the disease [182, 183, 186].

Yazawa *et al.* (1995) identified the *DRPLA* gene product in normal human brains as a $\approx$190 kD, and in DRPLA brains as a larger $\approx$205 kD [184].

Segregation distortion. In experimental organisms, segregation ratios deviating from those expected from simple Mendelian inheritance were occasionally observed. An example of selection in gametogenesis is found in *Drosophila*. The segregation-distorter (*SD*) locus has two known alleles. Normal segregation of chromosomes occurs with the wild-type allele. Males heterozygous for the mutant allele, under certain conditions of environment, show among progeny a marked departure from the expected 1 : 1 ratio in favor of the *SD* allele. This interaction of homologous genes during meiosis is called meiotic drive [74].

Relatively well-documented human examples of such an unusual segregation suggesting prezygotic selection may be congenital aniridia and Alport's syndrome which were reported by Shaw *et al.* in 1960 and Fuhrmann in 1963 respectively [187, 188]. Since that time, no particularly significant evidence for meiotic drive has been demonstrated for a long time. In 1990s, however, disease cases showing marked segregation distortion have successively been reported: retinoblastoma, cone-rod retinal dystrophy, split hand/split foot disease, myotonic dystrophy, DRPLA, and Machado-Joseph's disease [189-192].

In segregation analysis of DRPLA, Tsuji and his associates noted that the segregation ratios of mutant gene and wild-type gene of DRPLA were not equal in case of paternal transmission. However, such a distortion in segregation was not

Table 15. Segregation distortion observed in family data of DRPLA.

Parent of origin		Affected individuals	Unaffected individuals	Total individuals	Statistical test	
					χ^2	p
Male	Number	78	47	125	7.7	$p<0.01$
	%	63%	37%			
	M/F ratio	37.4	27.2	64.6		
Female	Number	36	50	86	2.3	$0.1<p<0.2$
	%	42%	58%			
	M/F ratio	22.1	24.3	46.4		
Number of total sample		114	97	211	1.4	$0.2<p<0.3$

M/F ratio: Male to female ratio. Ikeuchi *et al.* 1996 [185].

observed in case of maternal transmission (Table 15). These data suggest that the phenomenon of meiotic drive possibly occurs in man as well.

10. Genomic rearrangements

DNA segments in the human genome can move into new genomic locations or amplify themselves. Such genomic rearrangements may also have some effect on the expression of affected genes.

Some genomic rearrangements are unprogrammed, occurring randomly and thus have no physiological significance, such as transposon and movable genetic elements [193]. In some instances, however, they may cause mutations, modify the expression of the genes, or render tumor cells drug resistance by gene amplification. Others are programmed events that play a critical role in the control of expression of some genes during differentiation and the development of certain cell types.

Hozumi and Tonegawa (1976) first demonstrated that immunoglobulin genes rearrange during the development of B-cells. Variable regions of immunoglobulins are encoded by variable, diverse and joining gene segments that are located separately in the germ cell line, but brought together in B-cells by rearrangement. This mechanism generates the diversity of immunoglobulins required for recognizing almost unlimited number of foreign antigens [194, 195].

Honjo and Kataoka (1978) demonstrated that class switching and the successive change of immunoglobulin heavy chain also occur by gene rearrangements [196, 197].

Mak, Davis and their colleagues (1984) isolated genes encoding T-cell receptors, which possess the gene structure and strategy similar to those of immunoglobulins to create their diversity [198, 199].

The programmed genomic rearrangements are only known to occur in these antigen receptor genes of the immune system in man, but similar mechanisms can also conceivably operate in other cell types such as neural cells in the central nervous system where a great diversity may exist [200].

Allelic exclusion. During T-cell development, the site-specific DNA rearrangements mediating the assembly of β- and α-chain of the T-cell receptor are developmentally ordered. In particular, the assembly and expression of a complete β-chain gene blocks further rearrangements at the β-locus [201]. Such a phenomenon is referred to as allelic exclusion which is also observed in the process of rearrangements of immunoglobulins.

11. Approaches to multifactorial traits

Common diseases are mostly multifactorial. Such diseases are still not easy to analyze from genetic viewpoint, though Fisher showed, in a classical paper published in 1918, that such a mode of inheritance is an extension of, and not contrary to Mendelian concepts concerning unifactorial inheritance [15].

Table 16. Approaches to multifactorial traits in the years 1949-1965.

Stage I	The concept of polygenic system Mather 1949
Stage II	The concept of quasicontinuous variation Grüneberg 1952, Wright 1959
Stage III	The concept of threshold character Edwards 1960, Carter 1961, Falconer 1965

References: [21, 202-206].

Table 17. Specific association between HLA-B27 antigen and rheumatological diseases.

Disease	% Antigen frequency		Relative risk
	Patient	Control	
Ankylosing spondylitis			
Caucasian	90	8	87.8
Haida Indians	100	51	34.4
Bella Coola Indians	100	26	20.2
Japanese	67	0	305.7
Reiter's syndrome	78	8	35.9
Yersinia arthritis	79	9	24.3
Salmonella arthritis	67	9	17.6
Psoriasis arthopathica			
Peripheral	16	9	2.5
Central	40	9	8.6
Unspecified	42	8	7.1
Juvenile rheumatoid arthritis	26	9	4.1

Sasazuki *et al.* 1977 [207].

A. Developmental process of genetic approaches to multifactorial traits.

Genetic approaches to such multifactorial traits explored principally by English geneticists may chronologically be divided into three stages as shown in Table 16, and have now entered a fourth stage. The initiative of the fourth stage was the demonstration of association of a given trait with certain types of polymorphic traits.

HLA and disease association. The strongest HLA and disease association is that between ankylosing spondylitis and HLA-B27 antigen. As shown in Table 17, more than 90% of Caucasian patients with ankylosing spondylitis possess B-27, whereas only 5-9% of the controls possess this specificity. This strong association is also prominent in other ethnic groups. In Japanese, in whom B-27 antigen is extremely rare, ankylosing spondylitis again has a strong association with B-27 [207]. This means that the *HLA-B27* gene is major or submajor gene having pleiotropic effects on liabilities to rheumatological diseases as well as gene responsible for the production of HLA-B27 antigen. Such an approach is thought to be the starting point in the fourth stage.

B. Adult diseases

The adult diseases subjected to analysis in the recent past include the follow-

Table 18. Comparison of concordance for schizophrenia in MZ ad DZ twins.*

		Investigator				
		Kallman 1946	Slater 1961	Inouye 1961	Kringlen 1967	Allen *et al.* 1972
MZ	No. of pairs	174	41	55	55	95
	Concordance** (%)	69-86	68-76	60-76	25	27
DZ	No. of pairs	517	115	17	172	125
	Concordance** (%)	10-15	11-14	12-22	4	5
MZ/DZ ratio		5.7	5.4	3.5	3.8	5.7

* A fuller summary of twin studies is given in Allen *et al.* (1972), from which information in the above table was taken. ** The second figure is corrected for age. Sutton 1975 [74].

ing: diabetes mellitus, hypertension, obesity, neoplasma, osteoporosis, atherosclerosis, coronary heart disease, rheumatoid arthritis, atopic disease, allergic disease, inflammatory diseases of the intestine including Crohn's disease and ulcerative colitis, Alzheimer's disease, manic depressive illness, schizophrenia, alcohol dependency, *etc.*

Mental and psychiatric disorders. It is noteworthy that analyses have recently been directed toward mental and psychiatric disorders such as schizophrenia, manic depressive illness, Alzheimer's disease, and alcohol dependency which were previously thought to be out of reach regarding a determination of the pathogenesis.

In the case of schizophrenia, twin, family, and adoption studies have formed the basis of evaluation of the relative importance of genetic and nongenetic factors in the etiology [74, 208-211]. Several of the larger studies on twins are summarized in Table 18. These studies consistently show higher concordance rates for monozygotic twins as compared to dizygotic twins [74, 208, 209].

In a study of five Icelandic and two English families, with a total 39 cases of schizophrenia, evidence of linkage to RFLP markers for the chromosome 5q11-13 region was found [212]. However, an even more detailed analysis of this chromosomal region, schizophrenics showed no evidence whatever for linkage to this region. More recent studies have also failed to confirm this linkage [104].

Alzheimer's disease, on the other hand, is a representative example of mental and psychiatric disorders in which some progress has been seen. A combination of approaches has been used, including the rare example of families in which the disease appears to show a pattern of simple inheritance, cloning and analysis of candidate gene in pathways and the association of particular forms of a candidate gene with the morbid condition more often than expected under randomness [213]. A series of studies have thus started with great potentialities toward the future.

Diabetes mellitus. Diabetes mellitus is genetically and pathophysiologically heterogeneous, but there are at least two major types: insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). Twin

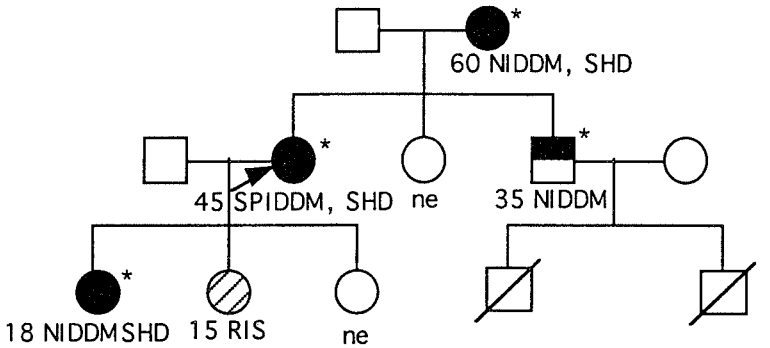

Fig. 4. A pedigree of mitochondrial diabetes mellitus (MIDD).
* Single base substitution at position 3243. SHD: sensory hearing disturbance, SPIDDM: slowly progressive IDDM, RIS: reduction of insulin secretion, ne: not examined. Kadowaki 1996 [219].

studies have suggested that hereditary factors play an important role in the both types, though they appear to be of much greater significance in NIDDM [214]. Most of IDDM and NIDDM are probably multifactorial, but changes in a few genes have been detected. The present section focuses only on such single-gene-determined entities of NIDDM on the basis of recent studies in Japan.

It was demonstrated that the secretion of a structurally abnormal insulin with a defective function (Insulin Wakayama) resulted in NIDDM, and that mutation in the insulin receptor gene causes also NIDDM due to impaired actions of insulin [215-217].

Oka *et al.* (1993) and Kadowaki *et al.* (1993) identified that single base substitution (an A to G transition) in the mitochondrial gene for tRNA [Leu(UUR)] at position 3243 causes NIDDM due to impaired insulin secretion to glucose [218-220]. This mitochondrial diabetes mellitus was maternally transmitted and often associated with sensorineural hearing disturbance. A family with this type of diabetes is shown in Fig. 4, where members showing slowly progressive IDDM and reduced insulin secretion are found [219]. This entity was thus called "maternally inherited diabetes and deafness" (MIDD), presumably comprising about 1% of all diabetics in Japan [218, 220].

Three genes are involved in the pathogenesis of maturity-onset in the young (MODY): genes for hepatocyte nuclear factor-4α, glucokinase, and hepatocyte nuclear factor-1α which were designated as *MODY1* gene, *MODY2* gene, and *MODY3* gene, respectively [221-225]. These are all thought to be responsible for the impairment of glucose-induced insulin secretion from β-cells of the pancreas.

12. Human cancer genetics

Numerous discoveries have been made on the molecular basis of oncogenesis

in man since the discoveries of the chicken sarcoma by Fujinami and Inemoto in 1910 and the sarcoma virus by Rous in 1911 [227, 228]. However, it is impossible to cover the whole subjects here in any depth. This section thus only outlines the major studies relevant to human cancer genetics.

Four categories of human cancers classified by conditions of familial occurrence. In discussing the role of hereditary and environmental factors in tumorigenesis, it is useful to classify human cancers into four categories: (1) rare genetic forms of cancer, (2) inherited common cancers, (3) cancer-prone families, and (4) common cancers [73].

The third category has been largely defined by Lynch as an autosomal dominant trait, tumors developing usually before the age of 40, and there are often multiple primary tumors at the sites of more than two organs [229]. At present this concept appears to be widely accepted, although it is still difficult to distinguish the apparent cancer-prone families from a familial aggregation of common cancers which could occur by chance [73].

Table 19. Major studies corresponding to milestones in human cancer genetics.

Discovery of Philadelphia chromosome and its chromosomal basis	Nowell & Hungerford 1960, Rowley 1973
Two hit hypothesis in tumorigenesis: *RB* gene	Knudson 1971, Cavenee *et al.* 1983
First recognition of human neoplasma due to a retrovirus—adult T-cell leukemia	Takatsuki *et al.* 1973, Poiesz *et al.* 1980, Miyoshi *et al.* 1979, Hinuma *et al.* 1981, Yoshida *et al.* 1984
Identification of oncogene	Shih & Weinberg 1982, Goldfarb *et al.* 1982, Parada *et al.* 1982, Santos *et al.* 1982, Reddy *et al.* 1982
Identification of proto-oncogene	Stehlin *et al.* 1976, Takeya & Hanafusa 1983
Identification of tumor suppressor gene	
RB gene	Friend *et al.* 1986
p53 gene	Lane & Crawford 1979, Linzer & Levine 1979
APC gene	Joslyn *et al.* 1991, Nishisho *et al.* 1991
BRCA1 gene	Miki *et al.* 1994
Association between growth factor and cancer	Doolittle *et al.* 1983, Waterfield *et al.* 1983, Downward *et al.* 1984
Signal transduction involving protein phosphorylation	Collet & Erikson 1978
Apotosis and cancer	Tsujimoto *et al.* 1984, Itoh *et al.* 1991

References: [69, 226, 230-257]. Compiled by J. Miyoshi (Takai Biotimer Project, ERATO/JST, Ohsaka).

Major studies corresponding to milestones in human cancer genetics. Table 19 lists the major studies corresponding to milestones in human cancer genetics which were conducted from 1960 to 1995 [226, 230].

Philadelphia chromosome. In a few neoplasias, a single specific chromosomal aberration limited to the tumor tissue has been described. The classic example is the Philadelphia chromosome discovered by Nowell and Hungerford in 1960, which is almost regularly associated with chronic myelogenous leukemia [69]. In 1973 Rowley identified that this chromosome occurs due to a reciprocal translocation between chromosome 22 and chromosome 9, with the break-point being in 9q34 and 22q11 respectively [231].

Retinoblastoma and the "two-hit" hypothesis. Most unilateral cases of retinoblastoma, a malignant tumor of the eye, are sporadic, but bilateral cases are often familial [232, 233]. In the latter cases the tumor occurs due to two mutational steps at the *RB* locus which is located on the long arm of chromosome 13 (13q14). Tumor formation only results when a second, and this time somatic mutation occurs in a retinal cell at the *RB* locus on the other homologous chromosome. In the former sporadic cases both first and second mutations occur in the same retinal cell. This "two-hit" hypothesis was proposed by Knudson in 1971, which accounts also for some cancers of familial occurrence [73, 233].

The first human neoplasma due to a retrovirus—adult T-cell leukemia. Around the year 1973, Takatsuki and his associates recognized the existence of adult T-cell leukemia (ATL), previously a disease of unknown entity [234]. A series of subsequent studies revealed this disease entity to be the first human neoplasma due to a retrovirus.

HTLV (human T-cell leukemia virus) corresponding to the pathogen of ATL was first reported by Gallo and his associates in 1980 [235]. They isolated HTLV from cultured cells taken from a patient with an aggressive variant of *Mycosis fungoides* and from another patient with Sézary's syndrome [234, 235]. Both diseases have been said to have cutaneous T-cell lymphoma, but they also showed some unusual features which, in retrospect, link them to the clinical entity of ATL. In Japan, the coculturing of ATL cells with umbilical cord blood lymphocytes was first done by Miyoshi *et al.* who obtained the cell line MT-1 [236]. Hinuma *et al.* demonstrated that ATL patients possess antibodies against presumed viral antigens on MT-1 cells [237]. Shortly after these studies, a retrovirus was isolated and named ATLV. Since Yoshida *et al.* showed that HTLV and ATLV are identical, the term "HTLV-1" (human T-lymphotropic virus type I) has commonly been used [238].

Identification of oncogene, proto-oncogene, and tumor suppressor genes. In 1982, a point mutation in a cellular oncogene was first reported independently by Weinberg and his associates, Wigler and his associates, and Barbacid and his associates in their studies of oncogene c-H-*ras* in human urinary bladder cancer [239-243].

Thereafter, two types of cancer-causing genes were recognized: proto-oncogenes and tumor suppressor genes. These are responsible for proteins whose normal function is to suppress uncontrolled cell growth and their inactivation results in the normal control being lost [244, 245]. Further studies have revealed the phenomenon called LOH and LOI, which have contributed to some of the mechanisms of both phenotypic expression of genes and gene mapping in man.

Loss of heterozygosity (LOH) and loss of genomic imprinting (LOI). The term "loss of heterozygosity" has been widely used in the field of human cancer genetics by providing a method for mapping chromosomal areas potentially containing tumor suppressor genes in different types of tumors (Table 20).

Genomic imprinting is also implicated in tumorigenesis (Table 21). An

Table 20. Loss of heterozygosity in tumorigenesis.

Neoplasma	Chromosome site of deletion (Gene involved)
Retinoblastoma	13q14 (*RB1*)
Wilms' tumor	11p13 (*WT1*)
Neurofibromatosis type 1	17q11 (*NF-1*)
Neurofibromatosis type 2	22q12 (*NF-2*)
Multiple polyposis of the large intestine	5q21 (*APC*)
Hereditary non-adenomatous cancer of the colon	2p16 (*MSH2*)
Breast and ovarian cancer	17q21 (*BRCA1*)
Malignant melanoma	9p21 (*CDKN2*)
Multiple endocrine neoplasia type 2A	10q11.2 (*MEN2A*)
Pancreatic carcinoma	18q21 (*DPC4*)
Angiomyolipoma	9q34 (*TSC1*)
Cancer of the large intestine	5q (*APC*, *MCC*), 8p, 17p (*p53*), 18q21 (*DCC*), 22
Small cell carcinoma of the lung	3p22 (*SCLC1*), 13q (*RB1*), 17p (*p53*)
Breast cancer	1p, 3p, 11p, 13q12 (*BRCA2*), 13q14 (*RB1*), 17p (*p53*), 17q21 (*BRCA1*)
Hepatic cell carcinoma	4q, 11p, 13q, 16q, 17

Compiled by J. Miyoshi (Takai Biotimer Project, ERATO/JST, Ohsaka). [226].

Table 21. Loss of imprinting in tumorigenesis.

Gene	Chromosome	Neoplasma
IGF2	11p15.5	Wilms' tumor, Beckwith-Wiedemann syndrome, glioma, cerebellum and medulloblastoma, hepatoblastoma, Ewing's sarcoma, ovarian cancer, breast tumor, cervical carcinoma, bladder carcinoma, lung cancer, esophageal cancer, leiomyosarcoma, rhabdomyosarcoma
H19	11p15.5	Wilms' tumor, cerebellum and medulloblastoma, testicular germ cell tumors, breast tumor, lung cancer, esophageal cancer, cervical carcinoma, bladder carcinoma
p57KIP2	11p15.5	Wilms' tumor, Beckwith-Wiedemann syndrome, soft tissue sarcoma, lung cancer

Compiled by J. Miyoshi (Takai Biotimer Project, ERATO/JST, Ohsaka). [226, 258].

extreme case involving the whole genome is the hydatidiform mole, an androgenetic placental tumor, in which two entire sets of chromosomes are of paternal origin. The opposite genome constitution is observed in a subset of ovarian teratomas which arise from parthenogenetically activated eggs. The phenotypes of these tumors fit well with the features of androgenetic and parthenogenetic mouse embryos, respectively [90]. It is widely accepted that either or both of the following two events are involved in tumorigenesis, namely: the inactivation of tumor suppressor or growth-repressing genes and the activation of oncogenic or growth-promoting genes [258].

13. Human gene mapping

A number of methods have been used in human gene mapping since an autosomal gene was first mapped in 1968 [259]: linkage test using family data, evaluation of the gene-dosage effect, analysis by somatic cell hybridization, karyotype analysis of chromosomal aberrations, determination of homologies of synthetic loci, linkage analysis using RFLP markers, radiation-induced gene segregation, *etc.* [178]. Among these methods, the linkage analysis has become more powerful since multiple DNA based genetic markers, based upon short tandem repeats, developed for all human chromosomes. In addition, it is noteworthy that advanced methods of localizing a gene on a particular chromosome such as *in situ* hybridization, fluorescent *in situ* hybridization, microdissection have recently become available (p. 274).

Two monumental studies of human gene mapping. The elucidation of the molecular basis for progressive muscular dystrophy was the first successful application of the technology of positional cloning to the isolation of dystrophin, the product of an X-linked gene.

The complete dystrophin cDNA, the largest known gene in man located on the short arm of chromosome X, was isolated by Kunkel and his colleagues in 1985. The isolation of the dystrophin gene has provided investigators with a lot of information on mutations which particularly involve the Duchenne and Becker types of muscular dystrophy [260, 261].

As compared with such X-linked muscular dystrophy, there remain many difficulties in mapping genes responsible for the diseases in which the gene products causing the diseases have not been isolated. In such diseases a certain amount of luck is required to obtain significant evidence for linkage between a particular gene and some genetic marker. Huntington's disease was such a successful case [104, 262, 263].

Gussella and his colleagues (1983, 1986) studied two large kindreds with many affected members using 12 DNA probes. One probe designated "G8," derived from a recombinant bacteriophage from a human gene library, was noted to detect two invariable and several variable *Hin*dIII fragments in human genomic DNA. It turned out that there were several polymorphic sites for this enzyme in

relatively close proximity so that the frequency of recombination between them was negligible, that is they were transmitted together as a unit [262, 263]. The haplotypes were designated A, B, C, and D. In a North-American family the A haplotype was associated with Huntington's disease; in a Venezuelan family it was the C haplotype. Subsequently, a DNA probe designated D4S10 was generated which was estimated to be four recombination units from the disease gene and which was assigned to the terminal band of the short arm of chromosome 4 [104, 262, 263].

Some improvements in the linkage analysis. In addition to a long-standing method such as the lod score method, affected sibpair method and location score method have developed in the linkage analysis [264], and the calculations in the analysis have not become difficult by using a computer program specifically designed for calculations.

The human genome project. Major projects in the medical biology that remain to be studied before the 21st century may be the following: high-dimensional functions of the brain, development (ontogeny), aging process, prophylaxis and treatment of cancer, automaticity of the heart, immunity and autoimmunity, *etc.* The completion of the human genome project will undoubtedly promote the solution of these major projects in the medical biology [265-267].

The human genome project, which started in 1989, has set itself three major scientific goals: creation of genetic maps, development of physical maps, and determination of the complete sequence of human DNA [268].

The goal in the first round of the human genome project may be the identification of the complete set of human genes and the sequencing of the entire human DNA in which the haploid genome consists of approximately 3×10^9 base pairs. In parallel to the human genome, the genome projects of organisms familiar to man will progress: mouse, drosophila, yeast, worms, bacteria, *etc.* [73, 178, 265-268].

Table 22. Development of the technology for accelerating human gene mapping.

1. *Development in laboratory procedure*
Positional cloning
Fluorescent *in situ* hybridization
Microdissection
Cloning of large DNA fragments using YAC or BAC chromosomes
Mapping based on cloning of human-mouse hybrid cells
In vivo experimental systems (transgenic animal, knock-out animal, *etc.*)
2. *Improved efficiency in laboratory procedure*
Improvement in the automatic DNA sequencer
Utilization of robots
Development of bioinformatics using computer systems
3. *Advance in international exchange of informations*
Improvement and development of gene library
Maintenance and compilation of data base on DNA fragments
Exchange of informations between investigators through the Internet Systems

It is estimated that the goal in the first round may be attained much earlier than previously expected due to the combined development of gene manipulation and computer technology as shown in Table 22. Among a number of excellent technologies, *in vivo* experimental systems such as transgenic animals and knock-out animals will be powerful tools particularly in the study of gene action [269-271]. The target date for the first round is presumed to be the years 2005-2010 [265-268].

A large proportion of genomic DNA remains for which at present there is no defined function, and due to mechanisms which we still do not comprehend, it preserves its own existence within the genome. However, this part of the DNA sequence will be identified to have significant functions as research progresses [73].

Mitochondrial genome. The DNA sequence of human mitochondrial genome was determined by Anderson and his colleagues in 1981, and thereafter 37 loci were mapped including about 17 kinds of mitochondrial disorders due to point mutations or deletions in mitochondrial DNA [259, 272].

The DNA Data Bank of Japan (DDBJ) is outlined in Appendix 3.

14. An ecogenetic problem in the present and future—man and microbes

The ecogenetics covers the study of genetically determined differences in susceptibility to the action of physical, chemical, and infectious agents in the environment [273]. Such differences in susceptibility may be either unifactorial or multifactorial in causation.

Among the ecogenetic problems with which man is confronted at present, this

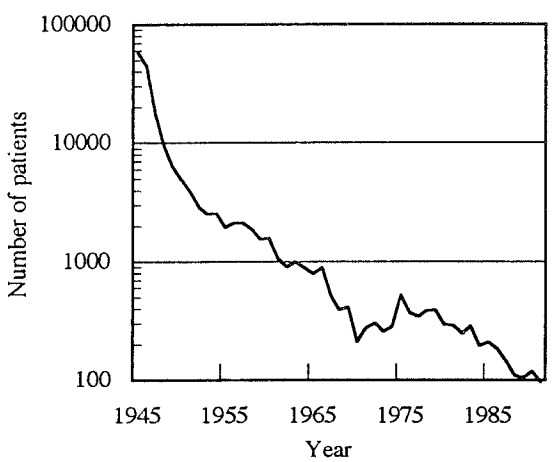

Fig. 5. Yearly change in the number of patients with typhoid fever in Japan. Reports from the Japan Association of Health & Welfare Statistics (1980, 1982, 19984, and 1992). [277].

section focuses on the interrelations between man and microbes which may be an important and serious issue in the future as well as in the present.

A. Change in infection in the past decades

Man's ability to control the environment has rapidly and remarkably increased with cultural development in the past hundred years [274]. One such manifestation of this trend is the ascendancy of infectious and contagious diseases.

In the decades following World War II, the campaigns against small pox and polio suggested that people could possibly rid themselves of world epidemics [275]. Antibiotics, vaccines, and sanitation cured infections that used to only be accepted as fate. As a result, neonatal and infantile deaths have been greatly reduced, and the average life span has dramatically increased.

At the time the eradication of small pox was declared in 1980, huge reductions were also seen in typhoid fever, a representative acute infectious disease designated by law in Japan (Fig. 5), and *Encephalitis japonica*, rabies, and other fatal infectious diseases had all nearly disappeared in Japan.

At this point of time, some human geneticists said that there was dramatic trend toward the control of the infectious and contagious disease entities, and as a result, an increasing proportion of medical attention will be forcibly directed toward those diseases in which the "host factor" or endogenous factor appears to be relatively more important.

Just a few decades later, on the other hand, it was difficult to appreciate such trends. Table 23 gives the infectious diseases showing strong virulence and infectivity which began to appear in the peak polio years, and then more and more erupted [275]. As when any new infection appeared, there still remained doubts as to whether it is new or has just been newly recognized, but they were almost unknown until the years described in the Table 23.

Table 23. A partial list of new diseases and the years of their first appearance or recognition. Isolated cases or localized epidemics of some bad occurred earlier.

Year	Infectious disease	Year	Infectious disease
1951	Korean hemorrhagic fever	1970	Human toxoplasmosis
1953	Dengue hemorrhagic fever	1975	Lyme disease
1953	Argentine hemorrhagic fever	1976	Ebola fever
1955	Chikungunya	1976	Legionnaires' disease
1956	Kyasanur Forest disease	1977	Adult T-cell leukemia
1957	Human babesiosis	1977	Rift Valley fever
1959	O'nyong-nyong fever	1980	Toxic shock syndrome
1960	Bolivian hemorrhagic fever	1981	AIDS
1961	Oropouche	1982	*Escherichia coli* O157: H7
1965	LaCrosse encephalitis	1984	Brazilian purpuric fever
1967	Marburg disease	1986	Human ehrilichiosis
1967	Intestinal capillariasis	1989	Venezuelan fever
1968	Pontiac fever	1989	Toxic-shock-like syndrome
1969	Lassa fever	1993	Hantavirus pulmonary syndrome

Karlen 1995 [275].

It may be fresh in the memory of most Japanese that a new strain (O157: H7) of the common microbe *Escherichia coli* prevailed in Sakai City and other parts of Japan in 1996, thus raising worries about the safety of the public supply of food.

In addition to the infectious diseases listed in Table 23, there are also a number of acute and chronic infectious diseases that have returned or again become wide spread after previously having been localized, limited, or controlled.

Man has made strenuous efforts to domesticate wild animals since the ancient times. Such efforts had resulted in an increase in opportunity that microbes are transmitted from animals to man. It is said that the man shares more than 400 kinds of microbes with domesticated and wild animals [275]. However, such a situation in zoonosis appears to change probably due to variations occurring in microbes.

B. Factors leading to disease emergence

Major factors leading to the emergence of infectious disease may be presumed as follows: an increase in the size of population with a higher density, rapidly increasing discrepancy between the size of population and the availability of the resources to support the population, changes in the environment on the earth with special reference to global warming caused by the burning fossil fuel and massive deforestation, development of scientific technology and industry, changes in human life style and behavior, extension of international communication by travel and trade, progress in medicine, microbial adaptation and change, *etc.* [275, 276, 278].

Among these factors, this section will focus on the factors of "microbial adaptation and change."

Drugs and medical technology. A wide variety of drugs have been introduced to medical practice by the rapid development of industry of synthetic chemistry over the past few decades. An increasing number of new drugs has led to increasing amounts of iatrogenic disorders. A research area of genetic variations in response to drugs—pharmacogenetics was thus established in the years from 1957 to 1965 as a subdivision of ecogenetics (xenogenetics), which covers the hereditary factors in the response of microbes to drugs as well as those in man [279-281].

Changes in the age structure in the population and the widespread use of immunosuppressants have also led to an increase in immunocompromised hosts. Infections of more than 1,100 kinds of opportunistic microbes have been noted with an increase in drug-resistant microbes at medical institutions [92, 93].

The development of medical technology, particularly involving invasive procedures has also resulted in an increased opportunity of infection in parallel to an increase in immunocompromised hosts, and the development of industrial technologies related to building equipment has also made legionellosis common around the world [275].

Microbial adaptation and change. Many microbes have shown mutational changes in their genomes that alter their infectivity, virulence, and resistance to environmental changes.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a mutant strain which began to prevail all over Japan in the early 1980s. Hiramatsu and his associates (1992) studied the genomic changes in MRSA strain N315. Molecular cloning and analysis of nucleotide sequence of this strain revealed that two open reading frames (orfs) were identified in the 5′-flanking region of the *mec*A gene. These orfs were thought to encode the putative co-inducer and repressor proteins specific for the regulation of methicillin resistance in MRSA [282].

In addition, it should also be kept in mind that a lot of drugs including antibiotics have also been given to domesticated animal species for food. Such customs also influence the precipitated resistance of microbes to drugs.

The genetically determined vital system in man, particularly involving the immune system, does not appear to have yet been exposed to a sufficient number of arrangements to respond to such changes in microbes.

15. Concluding remarks

According to a survey in 1993, progress in the treatment of inborn errors of metabolism was better than it had been, but it was still only a partial success. The advances were attributable to greater success with organ and tissue transplantation, better pharmacotherapy, and better support systems [283]. Restoration of normal homeostasis, the key to successful treatment, remains an elusive challenge and is a logical major focus for research in human genetics [283].

Since the first recombinant molecules were generated in 1972, great improvements have been made in the accuracy of risk estimation of hereditary diseases [73]. With the progress of the human genome project, people will soon be able to make more well-informed decisions in the risk estimation of hereditary diseases, and before long such common diseases as cancer, diabetes, Alzheimer's disease,

Table 24. Factors altering human reproduction.

Birth control
Artificial abortion
Assisted reproductive technology
In vitro fertilization and embryo transfer
Gamete intrafallopian transfer, zygote intrafallopian transfer, pronuclear stage tubal transfer, *etc.*
Micro-insemination
Embryo biopsy
Selective reduction of multifetal pregnancy
Surrogate mother
Reproductive engineering

Compiled largely based on Hiroi's article [288].

etc., will likely also be subject to genetic counseling.

Under these circumstances, the rights of people to remain ignorant of such risk for a given disease as well as the rights to know such risks should be ensured. More specifically, close attentions should be paid to the protection of such informations on the genetic constitutions of each individuals to preserve their privacy, and to obtain a nation-wide understanding and agreement on the application of DNA technology to the practice in medicine including gene diagnosis and therapy. A number of reports on the guidelines of genetic counseling, which will be required in the era of DNA technology, have recently been published [284–287].

Finally, the author would like to arouse the interest of the members of the Society to the recent trends in the technology pertinent to human reproduction as shown in Table 24. These technologies will sooner or later raise many new issues regarding ethical problems as well as various factors related to altering the genetic constitutions of human populations.

Appendix data

APPENDIX 1. Pioneers of human genetics in Japan.

Name	Year of birth and death	City	Name	Year of birth and death	City
Hisomu Nagai	1876–1957	Tokyo	Minoru Itoh	1894–1982	Sendai
Taku Komai	1886–1972	Kyoto	Riichi Kawakami	1895–1982	Tokyo
Seizo Katsunuma	1886–1963	Nagoya	Kunizo Fukuda	1896–1988	Tokyo
Tsunekichi Ueda	1887–1966	Nara	Yushi Uchimura	1897–1980	Tokyo
Shyungo Ohsato	1888–1974	Sendai	Shufu Yoshimasu	1899–1974	Tokyo
Yoshio Koya	1890–1974	Tokyo	Toratoshi Taniguchi	1902–1991	Tokyo
Tanemoto Furuhata	1891–1975	Tokyo	Sajiro Makino	1906–1986	Sapporo
Tando Misao	1893–1994	Fukuoka			

APPENDIX 2. List of studies and recipients of the Japan Society of Human Genetics Award (1960–1996).

Year	Name of recipient	Title of studies awarded
1960	Shigeo Takahara	Studies on acatalasemia
1961	Ei Matsunaga	Selection due to mother-fetus ABO incompatibility
1962	Ujihiro Murakami	Developmental mechanisms of congenital anomalies
1963	Hisatoshi Mitsuda	Clinical genetic studies in psychiatry
1964	Susumu Shibata & Akira Tamura	Studies on hereditary nigremia (Hb M Iwate)
1965	Shigeichi Sunahara	Pharmacogenetic studies of INH metabolism
1966	Toshiyuki Yanase	A study of isolated populations
1967	Sajiro Makino	Chromosome studies in human subjects
1968	Shoei Iseki	Biochemical genetic studies of blood group substances
1969	Eiji Inouye	Studies on twins
1970	Motoo Kimura	Theoretical studies of human population genetics
1972	Hideo Yamaguchi	Studies on *cis* AB blood group
1973	Toshiyuki Furusho	Genetic studies on the stature
1976	Katumi Tanaka	Genetic studies of inbreeding

Year	Name of recipient	Title of studies awarded
1977	Masatoshi Nei	Theoretical studies on molecular population genetics and evolution in man
1978	Yoshio Okada	Studies of somatic cell genetics utilizing cell hybridization
1979	Shiro Miwa	Erythrocyte enzyme system and its clinical and biochemical studies
1980	Susumu Ohno	Studies on sex determination in mammals including man
1981	Akira Yoshida	Biochemical genetic studies on human enzyme variation
1983	Keiya Tada	Studies on inborn errors of metabolism
1985	Tadashi Kajii	Studies on androgenesis of hydatidiform mole, and related studies
1986	Teruo Kitagawa	An animal model of human acid sphingomyelinase deficiency (Niemann-Pick's disease) and the study of its enzyme replacement
1988	Masao S. Sasaki	Cytogenetic studies on hereditary factors for oncogenesis
1989	Takehiko Sasazuki	Genetic control of immune response and disease susceptibility
1992	Kiyoji Tanaka	Molecular analysis of xeroderma pigmentosum group A gene
1993	Kazuya Mikamo	Cytogenetic studies of reproduction
1994	Ichiro Matsuda	Molecular aspects of inherited metabolic disorders of amino acids
1995	Yusuke Nakamura	Roles of DNA markers in medicine
1996	Tadao Orii	Molecular basis of β-ketothiolase deficiency, mucopolysaccharidosis and Zellweger syndrome

APPENDIX 3. **DNA Data Bank of Japan (DDBJ)**

The DDBJ DNA data bank was started in 1986 with the endorsement of the Ministry of Education, Science, Sports and Culture of Japan, and representative molecular biologists in Japan.

From the beginning it was intended that the DDBJ should be operated as one of the International DNA Databases including the Bioinformatics Institute (EBI) at European Molecular Biology Laboratories (EMBL) in Europe and the National Center for Biotechnology Information at the National Institute of Health in the USA (GenBank/KCBI/NIH) as the two other members. Consequently, the DDBJ has been collaborating with these two data banks mainly by daily exchanges of collected data at each bank through international networks, and by holding two annual meetings, the International DNA Data Bank Advisors Meeting and the International Data Bank Collaborators Meeting.

The DDBJ is the sole DNA data bank in Japan, which is officially certified to collect DNA sequences from investigators and which can issue accession numbers to the data submitters.

The DDBJ collects data mainly from Japanese investigators, but of course accept data and issue accession numbers to investigators in any other countries. It has been noted that since the DDBJ exchanges data with EBI and GenBank/NCBI on daily basis, the three data banks share virtually the same data at any moment.

The DDBJ also provides investigators worldwide with various on-line data retrieval services such as FASTA, BLAST, CLUSTALW, MALIGN and Key word searches. Please refer to:

http;//www.ddbj.nig.ac.jp for more information.

[Reference] Takeno Y & Gojobori T (1996) DNA Data Bank of Japan in the age of information biology. Nucl Acids Res. **25**: 14-17.

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