日本人類遺伝学会第 28 回大会特別講演要旨

Abstracts of Special Lectures, the 28th Annual Meeting of the Japan Society of Human Genetics

I. THE POLYMORPHISM AND THE EVOLUTION OF VITAMIN D-BINDING PROTEIN

Jacques Constans
(Centre d'Hémotypologie du CNRS—Toulouse—France)

Vitamin D is, with the calcitonin and the parathyroid hormone, and important factor in the regulation of the calcium and phosphorus metabolism. According to the degree and to the duration of the deficiency in vitamin D, several problems are observed concerning the body growth, the skeletal development (rickets). Some organs such as the kidney, or the intestinal mucosa are essential in the vitamin D metabolism. Higher animals including man are obviously under the dependence of this vitamin for a normal life. The first description of the vitamin D deficiency was made by Soranos of Epheus (100–200 AC) but documented descriptions have been available only since 1650 from the thesis of Pr. Francis Glisson. The relationship between the incidence of rickets and lack of sunshine and/or partial deficiency of a nutritional factor are now accepted although their respective influence is still not clearly shown and is a matter of speculations. Skin, liver, gut, bone and kidney are involved in the essential stages of the vitamin D metabolism in the production of the dihydroxylated and biologically active metabolites. It was, then reasonable to assume the existence of plasma carrier proteins. The presence of only one carrier protein seems accepted, today: but its precise biological role is still not fully elucidated. We postulate this activity as indispensable for life on the basis of the absence of any known total deficiency of the DBP.

It is obvious that investigation of the vitamin D metabolism requires a multidisciplinary approach.

It is in the anthropological and genetical fields that our knowledge of the DBP has most increased in these last five years. Discovered by Hirschfeld (1964), the Group-Specific-Component (Gc) polymorphism was limited to ten mutants (two frequent and 8 rare) in 1976 (M. Johnson). Today, 3 frequent and 78 rare mutants, and one exceptional mutant associated with a co-dominant autosomal protein deficiency, are referenced and classified.

The majority of the rare mutants are probably neutral mutations, the Gc^{1A1}, Gc^{1A2} and

Gc^{1A10} excepted. These rare mutants are a powerful tool for anthropological analysis:
* they have made it easier to follow and describe human migrations from prehistorical times and more recent inbreedings between groups. The distribution of these rare mutants reveals the population exchanges between North Africa and the Middle East regions, but also between Central Africa and West African Peulhs and Bantus.
* two African cradles are identified for the American Black people.
* migrations from India brought other mutants to Europe, and also to Bali and Java.
* in South Asia, the presence of at least three major influences can be assumed from the Gc polymorphism: the oldest proto-Austronesian speakers from South China followed by a North to South migration of the Tibetan-Sinitic groups and the aboriginal settlement in Australia, New Guinea and Micronesia.

On the opposite, the distribution of the Gc^{1F}, Gc^{1S} and Gc^{2} frequent alleles delineates large geographical areas overlapping the microdifferentiation obtained with the repartition of the rare mutants. A North to South cline of the Gc^{1F} and Gc^{2} allele frequencies is observed between the two hemispheres. This geographical distribution cannot be explained by gene diffusion and random drift only: one has to postulate that slight biological activities are probably present between the three Gc 1F, 1S and 2 proteins. We demonstrated, using analytical procedures, the existence of different affinities of these proteins for the vitamin D metabolites. In this respect, the biological activity of the DBP isoproteins would be to regulate the contributions of the vitamin D derivatives to the endocrine and target organs. At a population level, it is reasonable to consider the analogy between the geographical distribution of the skin pigmentation and the repartition of the frequent alleles.

In primates, DBP is also polymorphic in several groups, but only among the Hominidae species the characteristics of the DBP are closest to the human protein (Gc 1 isoproteins). Among other primate groups investigated (Ceboidea and Prosimi samples), the one band pattern is the usual (Gc 2 protein) picture. In the evolutionary step between primate and man, the rate of mutations seems to increase and new mutants such as Gc^{1F}, Gc^{1S} and Gc^{2} occur. They may have been fixed at the beginning by random gene drift. Their distribution, today, would correspond to the slight selective advantage discussed before.

The preservation of the affinity for the vitamin D metabolites implies the presence of two regions in the protein: one highly variable, ideal for phylogenetic analysis, the second, a constant one, not available for the same purpose would have been submitted to intense selective pressure preventing any significant mutation in the active binding site. The phylogenetic investigations demonstrate the existence of a DBP since the vertebrates appeared. The duplication of the ancestral DBP gene may have occurred over 500 M years ago and been followed by different mutations on the variable region. It must be noticed that the emergence of the vertebrates is associated with the development of the bone skeleton.

II. GENETIC MARKERS OF HUMAN IMMUNOGLOBULINS AND EVOLUTION

Erna van Loghem
(Central Laboratories of the Netherlands Red Cross Blood Transfusion Services, 1066 CX Amsterdam, The Netherlands)

The defense mechanism of man is governed by cellular and humoral immune responses. The proteins that exercise the humoral response are called immunoglobulins. These comprise a group of molecules with a common structure that consists of one pair of identical light chains and one pair of identical heavy chains.

The antibody function is localized in the variable part (V) of the molecule. The constant part (C) determines the (sub) type of the light chains and the (sub) class of the heavy chains.

In recent years insight in the organization of immunoglobulin genes and on the generation of antibody diversity was obtained. There are coding DNA segments (exons) separated by non-coding DNA segments (introns). The exons are recombined by splicing out the introns to a gene that codes for an integrated V-C polypeptide chain. The chromosomes that carry V and C exons are number 2 for kappa light chains, number 22 for lambda light chains and number 14 for heavy chains.

The products of the various exons can be distinguished by their antigenic determinants (epitopes). These are classified as idiotypes, isotypes, allotypes and isoallotypes.

Immunoglubulins are found in vertebrates from primitive fishes to man. Probably immunological surveillance was necessary to meet the risks of infection. The simplest molecules resemble mu chain polymers.

During evolution more genes for more specialized functions were developed by a process of gene doubling and duplication and subsequent mutation to the complex of genes that we know to-day and can recognize by their specific epitopes.

Occurrence of genetically determined epitopes (allotypes) on heavy and on light chains, means that there are alleles of the gene codings for this particular chain. The difference relates in general to mutation of one or two nucleotides.

Allotypes are known for three of the four IgG subclasses (Glm, G2m and G3m), for alpha 2 heavy chains (A2m), for epsilon chains (Em) and for kappa light chains (Km).

The heavy chain allotypes are inherited in fixed combinations called haplotypes. When various populations are compared there are striking differences in composition and in frequency of their prevalent haplotypes. Some haplotypes being characteristic for a particular race.

Investigation of families and of populations for immunoglobulin allotypes can give information as to their origin and migration. Typing of non human primates shows phylogenetic relationship.

III. STUDIES ON THE MARKER GENE, Gm st, CHARACTERISTIC OF MONGOLOID POPULATIONS

Hideo Matsumoto
(Department of Legal Medicine, Osaka Medical College)

Since 1962 we have investigated various aspects of the genetic markers of human immunoglobulins in our laboratory. Today I would like to focus my talk on the marker gene, Gm st, which characterizes Mongoloid populations.

I. Human immunoglobulins and genetic markers

Antibodies which play an important role in the immune response are termed immunoglobulins. In man, five major classes of immunoglobulins have been identified, (IgG, IgM, IgA, IgD, and IgE), each with its own characteristic structure and distinct functional role. All are comprised of regular aggregates of two types of polypeptide chain, one being the light chain and the other the heavy chain. The light and heavy chains are divided into two portions, a variable and a constant region, on the basis of differences in their primary amino acid sequences. The variable regions consist of aminoterminal sequences (residues 1 to 108) of the light and heavy chains. These variable regions, in which the amino acid sequences vary considerably, are the parts of the molecule which determine the diversity of antibody specificity. On the other hand, the constant regions of IgG, span residues 109 to 214 of the light chain and to 446 of the heavy chain.

Immunoglobulins of the IgG class can be divided into four isotypes, IgG1, IgG2, IgG3, IgG4, on the basis of amino acid differences which occur in the heavy chain constant region. These structural differences result in distinct antigenic determinants that can be detected serologically and form a series of allotypic or Gm markers that reflect genetically controlled polymorphisms, which most of this survey was dedicated to.

The Gm system provides unique markers in studies of human genetics, especially for the characterization of a particular population and in studies of genetic drift and gene flow, by the presence of either a unique haplotype in different races or by marked differences in the frequencies of identical haplotypes in the same ethnic groups. So far it has been shown that the Gm haplotypes common to Mongoloid populations are Gm ag, axg, ab3st, and afb1b3 and among Caucasoids ag, axg, and fb1b3 and among Negroids ab1b3,

ab1b3c and ab3s. Thus, Mongoloids are characterized by having ab3st and afb1b3 haplotypes and Caucasoids by fb1b3.

II. Discovery of the Gm st gene which characterizes Mongoloid populations

In 1964 when only Gm(a), (x), (f), (b1), (b3), and (c) had been described, we discovered a new Gm allotype (later designated Gm(t)) in studies of polymorphic traits of blood in the isolated population of Nose village of the Osaka prefecture. At the same time another new allotype (later designated Gm(s)) was found by Dr. E. van Loghem in the Netherlands. These two new genetic determinants were investigated and described together. The presence of the Gm st gene was confirmed by segregation patterns in family studies in Japanese and it was subsequently confirmed that the Gm st gene is a marker gene which characterizes Mongoloid populations.

III. Determination of the Gm phenotypes and haplotypes in Japanese

We have continued investigations of Gm allotypes in Japanese populations and families to determine the phentoypes and haplotypes in Japanese in addition to other Gm allotypes following the discovery of the Gm st. It was documented for the first time in 1968 that nine Gm phenotypes observed in the Japanese population can be accounted for by the presence of four Gm haplotypes, ag, axg, ab3st, and afb1b3. The presence of the four haplotypes was confirmed by segregation studies in families. We then investigated the distribution of Gm allotypes among 11 Japanese populations in various regions from Hokkaido to Okinawa. A test for heterogeneity of the data for the 11 samples clearly indicates homogeneity. The homogeneity of the Gm haplotype frequencies among the Japanese populations is striking although samples were obtained from populations covering a range of approximately 2,200 km from Hobbaido in the north to Okinawa in the south.

IV. Interaction between normal human IgG3 carrying Gm(st) and Protein A Sepharose CL-4B

After the discovery of the Gm st marker gene in Mongoloid populations, a few researchers have attempted to obtain IgG3 myeloma protein carrying Gm(st) in order to analyze the primary amino acid sequence. We have also been eager to analyze it, and have concentrated on obtaining this protein for ten years. However, it has proved difficult to obtain this protein for analysis because of the rarity of myeloma protein, since less than 1% of IgG3 myelomas would be expected to carry the Gm(st) determinants.

Protein A, which is a cell wall constituent of the *Staphylococcus aureus*, binds to certain IgG immunoglobulin subclasses. The IgG3 subclass protein is known not to bind exceptionally with the Protein A. Thus, the IgG3 subclass can be isolated from normal human serum using Protein A-Sepharose CL-4B immunosorbent. Seven years ago, we embarked on the isolation of IgG3 subclass protein carrying Gm(st) from normal serum based on binding capacity to Protein A in order to determine the primary sequence of the IgG3-Fc

fragment. It was shown in these studies that the IgG3 protein carrying different Gm allotypes from Japanese varied in its binding capacity to Protein A. On the basis of these results we postulated that the variability in binding capacity observed in our experiments had arisen either from amino acid substitution in the Gm allotypes or from a conformational change in the IgG3-Fc portion. The Fc portion of IgG3 molecule carrying Gm(st) allotypes might have the same binding site or conformation as those of IgG1, IgG2, or IgG4 subclasses. On the other hand, the lack of reactivity with Protein A may be due to a distinct structural difference within the Fc portion of IgG3 carrying Gm(b1b3), or Gm(g) allotypes.

A ^{1}H NMR study of the Fc region of human IgG1 and IgG3 immunoglobulins clearly showed that IgG3 carrying Gm(st), which was isolated from a Japanese patient with essential cryoglobulinemia (mentioned later), has histidine at position 435 as in the case of IgG1. We also confirmed that IgG3 protein carrying Gm(st) reacts strongly with Protein A. In marked contrast, IgG3 carrying G3m(g) does not bind Protein A. These results showed that binding of Protein A to the Fc region is not subclass-specific but that the presence of His-435 is necessary for binding of Protein A. However, it was to our regret that attempts to isolate IgG3 from normal serum using Protein A proved unsuccessful.

V. The primary sequence of the IgG3 myeloma protein (Jir) bearing the allotypic marker Gm(st) characteristic of Mongoloid populations

As I mentioned earlier, we considered it very unlikely that we would be able to obtain sufficient IgG3 for the analysis of the primary sequence. However, fortunately we were provided with IgG3 protein bearing Gm(st) which was cryoglobulin derived from a 57-year-old Japanese male with essential cryoglobulinemia treated by plasmapheresis. Thus, our hopes became reality. We were able to define the amino acid substitutions which determine the G3m(s) and G3m(t) specificities, characteristic of Mongoloid populations, by sequence analysis of the Fc region of this myeloma protein. By comparing the amino acid sequences of the IgG3 (Jir) and the other IgG subclasses which have been analyzed to date, we established the amino acid substitutions determining the G3m(s) and G3m(t) specificities characteristic of Mongoloid populations. It was found that G3m(s) is an isoallotype specified by an amino acid substitution at position 435, *i.e.*, whereas IgG1, IgG2, and IgG4 each have histidine in common, G3m(s−) had arginine in this position. This was also confirmed by the observation that the Fc fragment in question binds to Protein A. It was also established that the amino acid at position 379 of G3m(t−) IgG3 and the other subclasses is valine, whereas methionine in this position is specific for G3m(t+). Thus, the Gm st gene which characterizes Mongoloid populations defines on IgG3 subclass which has a histidine substitution at residue 435 and methionine substituted at position 379.

VI. Mongoloid populations from the viewpoints of Gm patterns

Taking into account that as a characteristic of the Gm allotypes as genetic markers, the difference among races in the Gm system are qualitative; *i.e.*, some haplotypes are absent from or confined to certain races and that marked differences in haplotype frequencies are observed from population to population within the same race, the distribution of Gm allotypes has been investigated in our laboratory using a series of serum samples from various ethnic groups from different regions in Southeast Asia through East Asia into South America.

The data indicate that three to nine Gm phenotypes which are accounted for by the presence of either three or four of the four haplotypes, Gm ag, axg, ab3st, and afb1b3 which are characteristic of Mongoloid populations were observed in these populations. A surprising heterogeneity in Gm haplotype frequencies was also detected. A striking aspect of the Gm data obtained from Mongoloid populations is the presence of a clear genocline, particularly for the Gm ag and Gm afb1b3 haplotypes from Southeast Asia through East Asia into South America. The Mongoloid populations can be divided into two groups; one is a "Northern group" which is characterized by a high frequency of the Gm ag haplotype and an extremely low frequency of the Gm afb1b3 haplotype and the other a "Southern group" is characterized by a remarkably high frequency of Gm afb1b3 and a low frequency of Gm ag. A genocline was also found for the Gm ab3st haplotype, which is a marker gene for Mongoloids, from Korea and Japan to Southeast Asia and from Alaska to South America. Thus, there seems to be sufficient evidence to assume the existence in the past of two distinct Mongoloid populations among the paleo-Mongoloid populations of East Asia. The degree of inter-population differentiation approximated for each haplotypes using the fixation index (Fst) may provide evidence that the differences in Gm haplotype frequencies, especially for Gm ag and afb1b3, is due to factors other than random genetic drift such as gene flow and natural selection.

I have waited a long time to be able to complete the last chapter of "The Gm st story" by my recent visit to Siberia. The project to "Genetic and Anthropological Studies of Mongoloid Populations in Siberia, in USSR" by a collaboration between Japanese and Soviet scientists was started in September this year. The serum samples from five regions, Kamchatka, north Baikal, south Baikal, Mongol, and Uralian were provided after my visit to Ulan-Ude on the Baikal Lake coast in Siberia where I had been eager to visit. Among those, the samples from north and south Baikal are from Buriats who are considered to have prominent Mongoloid characters. Included are three populations from Mainland China provided by my friend, Dr. Schanfield and includes unpublished data on Mongol population in China, I would like to discuss the results from 24 Mongoloid populations.

The differentiation of the Northern and Southern groups of Mongoloid populations on

the basis of the genocline of Gm ag and Gm afb1b3 haplotypes, as I mentioned before, will become more sophisticated as a point of contact in Mongol by the addition of these eight populations. In conclusion, I should like to say that the Gm st gene which characterizes Northern Mongoloid populations was found in the highest incidence in the northern Buriats who are considered to have the most prominent Mongoloid characters and the gene flows in all directions. However, the Gm st gene is found still in rather high incidences among Alaskan Eskimo, Koryak in Kamchatka, Mongol in USSR, Korean, Japanese, and Ainu in Hokkaido. Then a precipitous drops occur from North America to South America and from Mainland China to Southeast Asia. On the basis of the results on Gm haplotypes, which is the genetic markers of human immunoglobulins, I should point out that the Japanese race belongs to the Northern group of Mongoloid populations and the root of the race must exist in Siberia, most likely in the Baikal area.