日本人類遺伝学会　第32回 大会シンポジウム講演要旨

Abstracts of the Symposium, the 32nd Annual Meeting of the Japan Society of Human Genetics

Symposium. Medical and Biological Significance of DNA Polymorphism
シンポジウム. DNA 多型の医学生物学

S-1. DNA 多型の分子進化学的意義：多型の生成機構としての遺伝子変換：宮田　隆 (九大・理・生物). **DNA POLYMORPHISM AND MOLECULAR EVOLUTION: GENE CONVERSION AS A MECHANISM FOR GENERATING POLYMORPHISM.** Takashi MIYATA (Dept. Biol., Fac. Sci., Kyushu Univ., Fukuoka)

DNA の多型を生成する機構としてはすでにいくつか知られているが，ここでは分子進化機構の一つとしてよく知られている遺伝子変換 (gene conversion) をとりあげ，可能な多型生成機構の一つとして考察を加えた．

遺伝子変換とは，ある遺伝子の配列が，それと相同な他の遺伝子の配列で置き換わる現象のことをいい，遺伝子重複によって生成された多重遺伝子族のメンバー間にしばしば見られる普遍的進化機構の一つである．遺伝子変換は本来異なる遺伝子間で配列を一様にする遺伝的機構であるが，しばしば変換を受ける領域が遺伝子の一部（多くの場合エクソン単位）に限定されることがある．この場合，変換を受けた側の遺伝子は一挙に多数の点突然変異を受けたのと同じ効果を持ち，多様化が急速に進む．部分的遺伝子変換が起きると，多数の塩基あるいはアミノ酸置換がある限定された領域にかたまって存在する，という特徴的置換パターンを示し，このパターンが MHC クラス I 遺伝子群にみられる多型の特徴と酷似していることから，遺伝子変換がその主要な生成機構として働いている可能性があることを指摘した．

つづいて塩基配列データから部分的遺伝子変換を検出する方法を実例によって示した．遺伝子のコーディング領域の同義座位やイントロンといった diverge した領域では，通常，遺伝子に沿ってホモロジーの程度がほぼ一様であることが，これまでの多数の遺伝子の解析から明らかにされている．もし同一染色体上に隣接する相同遺伝子間で，部分的遺伝子変換が起きていなければ上記の置換パターンが期待される．一方，もし部分的遺伝子変換が起きると，両遺伝子間のホモロジーが遺伝子に沿って不連続になる．ところで遺伝子変換は遺伝的組み換え現象の一種であるので，異なる生物由来の相同遺伝子間には起こらず，したがってホモロジーの不連続性は観察されない．こうしてホモロジーの不連続性が検出された遺伝子対と相同な他種生物由来の遺伝子間での置換パターンを比較することで，遺伝子変換を明瞭に観測できることを述べた．また遺伝子変換が起きた時期に関しては，比較した遺伝子間のホモロジーの程度から直ちにそれを推定できるとは限らず，第3の遺伝子が関与する場合には現時点で遺伝子変換が起きたと考えることも可能である点も指摘した．

S-2. DNA 多型と集団遺伝学. 高畑尚之 (遺伝研・集団遺伝). **DNA POLYMORPHISM AND POPULATION GENETICS.** Naoyuki TAKAHATA (Dept. Pop. Genet., Natl. Inst. Genet., Mishima)

種内あるいは種間で観察される相同な DNA 部位における配列上の差異は，DNA 進化の機構を解明する上だけでなく，その進化の歴史の復元やそれをサンプルした集団間や生物種間の系統関係を解明する上でも重要な情報である．わが国でも，a) 分子進化の機構論（中立突然変異浮動仮説 M. Kimura, 1968）の提唱，b) さまざまな遺伝子の塩基配列の比較に基づいた分子系統樹の作成，c) これまで予想できなかった遺伝子進化の新しい要因を明らかにした多重遺伝子族やウイルスに関する分子進化学的・分子集団遺伝学的研究，d) 近縁種とくにヒトを含めた霊長類の系統関係を解明する試みなど，多くの優れた業績があり現在も活発な研究が進められている．

本講演では，DNA レベルで観察される種内・種間変異に関するいくつかの話題を「遺伝子系図学」の立場から論じてみたい．遺伝子系図学とは，サンプルした相同 DNA 部位や相同な遺伝子間の近縁関係をあからさまに考察する集団遺伝学の一分野であって，それ自体興味のある重要な問題を取り扱うばかりでなく，さまざまな集団遺伝学の理論的結果を導き出すときにもたいへん有効な方法である．

遺伝子系図学の前提ともいえる一つの主張は，相同遺伝子間の系図はこれをサンプルした種の系統関係と必ずしも一致しない点にある．とくに比較している生物種が近縁であればあるほど，この不一致の確率が高くなる．このことを人種の起源に関する Allan Wilson グループの研究，キイロショウジョウバエ属の系統関係に関する石和グループの研究，ヒト・ゴリラ・チンパンジーの系統関係に関する分子系統学的研究などを例に取りながら紹介してみたい．

S-3. MITOCHONDRIAL DNA POLYMORPHISM IN HUMAN POPULATIONS. Satoshi HORAI (Natl. Inst. Genet., Mishima)

A restriction enzyme analysis of mitochondrial DNA (mtDNA) polymorphism in three Japanese populations (Shizuoka, Okinawa, Aomori) revealed that the frequencies of enzyme morphs and the distribution of restriction types were considerably different from each other. A phylogenetic analysis, however, showed that the clustering pattern of the constructed tree for the three populations was essentially similar to each other. Namely, in each tree, there are at least two distinct groups with different frequencies: A group with the smaller frequency (group I) first diverged from the other group with the larger frequency (group II). However, the frequencies of individuals belonging to group I are different among the three populations. The divergence time between the two groups was estimated as early as about 125,000 years ago, assuming the rate of mtDNA divergence as 2×10^{-8} per site per year. However, this dating seems too early, if we accept that racial divergence took place about 120,000 years ago, as estimated from gene frequency data for blood proteins (Nei and Roychoudhury, 1974). The tree constructed on the basis of the combined data for the three populations indicated that the mitochondrial DNAs appear to have been already polymorphic even in the founder population of Japan.

As mentioned above, the phylogenetic trees in the three local populations indicate that there are at least two distinct groups of mtDNAs in Japan. To evaluate the evolutionary significance of this finding, we constructed a phylogenetic tree of mtDNAs among the three major races, combining our data with those for Caucasians and Negroes analyzed by Cann (1982). A total of 117 restriction types were observed among the three racial groups. The three groups do not share any restriction types in common. The average number of nucleotide substitutions for Japanese is almost the same as that for Caucasians, whereas the value for Negroes is about two times larger than those for the other two groups. This implies that the mtDNAs of Negroes are much more diverse than those of Caucasians or Japanese. We also estimated the number of nucleotide substitutions for all pairs of individuals in the three racial groups and constructed a phylogenetic tree for the 117 types by the UPG method. We tentatively classified all lineages into eight clusters designated as C1 to C8. A phylogenetic tree showed 5 distinct clusters (C1, C2, C4, C5 and C6) specific for the particular racial groups, while in 3 clusters (C3, C7 and C8) there were interminglings of types from different racial groups. Most of the interminglings were likely to be due to gene migrations. However, the overall clustering pattern of the tree suggested that the ancestral human population has already been polymorphic in the mitochondrial genome before the divergence of the three major races.

Based on the above mentioned observations, the mtDNA analysis for patients of mitochondrial diseases were applied using denaturing gradient gel electrophoresis to detect a single mismatch of mtDNAs.

S-4. POLYMORPHISM OF GENES WITHIN HLA CLASS II AND CLASS III REGIONS. Takehiko SASAZUKI, Akinori KIMURA, Kikuo TSUKAMOTO, Fumiki HARADA, Kazunori URABE and Kenji HIRAYAMA (Dept Genet., Med. Inst. Bioregul., Kyushu Univ., Fukuoka)

HLA on human chromosome 6 is composed of class I, II and III, genes, of which class I and II genes belong to the immunoglobulin super gene family. Whereas genes for immunoglobulin and T cell receptor within the super gene family utilize a mechanism of gene rearrangement in the differentiation of immune competent cells to gain an enormous degree of diversity, genes for HLA class I and II do not utilize this mechanism. Extreme polymorphism of HLA, however, is well demonstrated which gives extreme diversity of HLA in human population but not in individuals.

Southern blot analysis using HLA class II genes, DR, DQ and DP as probes, revealed that the degree of polymorphism of class II is much greater than that detected by classical serology. For instance, HLA-Dw2(DR2-DQw1) and HLA-Dw12(DR2-DQw1) haplotypes were undistinguishable by serology, but they show a strong response in the mutual

mixed lymphocyte reaction (Sone *et al.*, *J. Immunol.* **135**: 1288, 1985), and furthermore they differ in controlling the immune response to schistosomal antigen; namely Dw2 haplotype controls high response to schistosomal antigen whereas Dw12 controls non response to that antigen (Ohta *et al.*, *J. Immunol.* **131**: 2524, 1983; Hirayama *et al.*, *Nature*, **327**: 426, 1987). These observation indicated that HLA-Dw12 haplotype may differ in genes coding for DR2 and DQw1.

Southern blot analysis clearly revealed the difference in DR and DQ patterns between HLA-Dw2 and Dw12 haplotypes. HLA-DQw1 β gene from Dw12 haplotype was cloned and its base sequence was determined (Tsukamoto *et al.*, *Immunogenet.*, **25**: 343, 1987). The amino acid sequence of HLA-DQw1 β chain of HLA-Dw12 haplotype was deduced from the base sequence and compared with that of HLA-DQw1 β chain from HLA-Dw2 haplotype. Out of 11 amino acid differences between HLA-Dw2 and Dw12 haplotypes in the extra cellular domains, 10 were in the first domain especially around the amino acid residue 70 constructing a hypervariable region. More interestingly DQw1 β chain from Dw12 has extra 8 amino acid residues in cytoplasmic domain compared to that from Dw2. The extra 8 amino acids were corresponding to the 5th exon of the DQw1 β gene. The 5th exon was not utilized in DQw β genes from Dw2, 3 and 4 haplotypes due to the point mutation at the splicing site for the 5th exon. Among the class II genes in humans and mice, only HLA-DQ shows this mutation, and among the DQ genes there is a polymorphism in this sequence. These differences in structure observed between Dw2 and Dw12 haplotypes may explain the difference in controlling the immune response to schistosomal antigen between Dw2 and Dw12 haplotypes.

Genes for the fourth component of complement (C4) and for the 21-hydroxylase (21-OHase) are located in the HLA-class III region. Two sets of C4 and 21-OHase genes are tandemly located; C4A-21-OHase A-C4B-21-OHase B, and there is a strong (more than 97%) homology between 21-OHase A and B genes. However, 21-OHase A is a pseudogene due to the presence of terminating codon within coding region. During the molecular analysis of a patient with 21-OHase deficiency, we found that conversion of 21-OHase B gene to A gene is the mechanism for the 21-OHase deficiency (Harada *et al.*, *Proc. Natl. Acad. Sci. USA.* **84**: 8090, 1987), which can be detected by Southern blot analysis. Using this information, extensive analysis of the class III region was performed and it was revealed that the partial conversion of 21-OHase A gene to B gene occurs in normal individuals suggesting that the bidirectional gene conversion or gene conversion-like event operates in this region.

Dynamic genetic events observed in the HLA region may explain the diversity and polymorphism characteristic to the genes within this region, and may also explain the normal and abnormal functions with great diversity governed by the genes in the HLA region.

S-5. MOLECULAR DIAGNOSIS USING RFLPs: A CLINICAL APPLICATION TO EARLY DETECTION OF FAMILIAL HYPERCHOLESTEROLEMIA.

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Haplotype analysis using RFLPs should be useful for the molecular diagnosis of many monogenic disorders, because most single nucleotide changes in the genome are not demonstrated readily by Southern blotting analysis and because most genetic diseases are heterogeneous at the molecular levels. Familial hypercholesterolemia (FH) is a common autosomal dominant disorder and results from a mutation in the LDL receptor gene. Since most cases of FH are due to inherited mutant alleles rather than new mutation and since the genetic heterogeneity seems to be common in FH, haplotype analysis using RFLPs should be useful for the early diagnosis of FH. The rationale for the early diagnosis of FH and the use of molecular diagnosis in FH is the following: 1) Drug and dietary therapy together with no-smoking are effective for the prevention of premature coronary heart disease in individuals with FH. 2) Tendon xanthomas, the detection of which is crucial for the clinical diagnosis of FH, do not appear before the latter part of the second decade and are variable in expressivity even among adult cases of FH.

We have analyzed the LDL receptor genes of 67 unrelated healthy Japanese and 38 members of six consecutive families with FH, by Southern blotting analysis using LDL receptor cDNA fragment probes. Seven RFLPs with relatively high PIC values have been detected at the LDL receptor locus in the 67 unrelated subjects. They include RFLPs at the site for *Taq*I, *Ava*II, *Apa*LI/I15, *Pvu*II, *Nco*I, *Pst*I, and *Apa*LI/3'. The heterozygosity of the haplotypes for the seven RFLPs was 0.88 in the 67 unrelated subjects. As to the mutant LDL receptor genes in the six families, Southern blotting analysis demonstrated readily the presence of a partial deletion in the gene in two families. Further analysis using LDL receptor cDNA subfragment probes revealed that a deletion including exon 15 or 16 was present in the mutant gene in one of the two families and that a deletion including exons 16 to 18 existed in the mutant gene in the other family. In three of the remaining four families, the haplotypes of RFLPs were informative for the identification of the mutant LDL receptor genes. The haplotypes of the mutant genes were different from one another among the three families. In the remaining one family, the proband was homozygous for all the seven RFLPs but an unusual *Taq*I 1.5 kb band served as a DNA marker for the detection of the mutant LDL receptor gene in this family.

These data suggest that molecular diagnosis using RFLPs is useful for the early detection of FH and that the origin of mutant LDL receptor genes tends to vary with pedigrees with FH in Japan. The data also indicate that more RFLPs must be revealed at the LDL receptor locus to increase the PIC value.

S-6. POSSIBLE RACE-ASSOCIATED PATTERNS OF RFLPs AND IMPLICATION IN THE CLINICAL APPLICATIONS OF THEM. Yasuo NAKAGOME (Dept. Cong. Abn. Res., Nat. Child. Med. Res. Ctr., Tokyo)

RFLPs have proven themselves to be useful in the prenatal diagnosis of metabolic disorders such as phenylketonuria and factor VIII deficiency. However, the applications are not limited to the diseases with known biochemical abnormalities. RFLP-study has also made it possible presymptomatic and/or prenatal diagnosis of Huntington disease, Duchenne muscular dystrophy, von Recklinghausen disease, manic depressive syndrome, tuberous sclerosis and at least ten other diseases. RFLP diagnosis needs probes as well as information on their PIC (polymorphism information content). Nearly all the probes available so far have isolated in either United States or Europe and thus most information on them were based on Caucasians.

In the present study, 38 different RFLP probes were applied on a presumably normal 50 to 60 Japanese. Some of the probes were combined with two or three different enzymes, *i.e.*, a total of 43 sites were analysed. In 5 of them, no polymorphic pattern was detected among Japanese although our own study and those in the literature revealed polymorphism among Caucasians. In additional 4, a new RFLP pattern which has not been described in Caucasians was observed. Again, our own study on some 10 Caucasians revealed similar results to what was described in the literature. The new RFLP pattern could be unique to either Japanese or Orientals, although further studies are needed. In additional 8 sites, incidence of two groups were very different. For example, a probe detected both A1 and A2 fragment at 95:05 ratio among Japanese, whereas what was described in Caucasians was 71:29, thus dropping PIC from 0.33 (Cauc.) to 0.09 (Jpn.). Using probes with high PIC, a linkage study on a familial neuro-muscular disease has just been launched.

As to oncogenes, we detected two RFLP sites within the *N-myc* gene. No RFLP has been described in the literature within it. An RFLP pattern of amplified *N-myc* in 6 cases of neuroblastoma and a case of rhabdomyosarcoma has been analysed. In all of them, a 17 kb *Sph* I fragment (S2 pattern) was detected. In the normal population, the "gene" frequency of the fragment was about 50%. Whether the *N-myc* with the S2 pattern has a tendency to amplify is being examined.

S-7. DNA 多型と human gene mapping. 吉田迪弘（北海道大・理・染色体研）. **DNA POLYMORPHISM AND HUMAN GENE MAPPING.** Michihiro C. YOSHIDA (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo)

ヒトの遺伝子数は5万ともいわれているが，現在までにわかっている種々の形質や遺伝疾患などの種類は4,000以上におよび，そのうちの約58%が常染色体性優性，35%が常染色体性劣性，7%が伴性遺伝のX染色体上の遺伝形式を示すとされている．これらの遺伝子の染色体上へのマッピングは

体細胞遺伝学，遺伝子クローニング技法などの発展によりここ数年の間に著しい進展をみている．とくに，DNA を制限酵素で切断した場合の多型性が染色体マッピングに有効であることが見いだされて以来，数多くの多型を示す DNA マーカーが分離され，このようなマーカーを家系分析に利用することにより，原因不明の遺伝疾患の遺伝子を追究する上で重要な手段となっている．

DNA やクローン化された遺伝子の多型を用いたリンケージ分析は現在つぎのような strategy により進められている．

A) 検索に必要なプローブの作成：1987年9月にパリで開かれた HGM9 でまとめられた多型を示す DNA マーカーの総数は750を超えるが，リンケージ分析をする上でより多型性を示す DNA マーカー，たとえば multi-allelic マーカーとして VNTR (variable number of tandem repeat) プローブなどの開発・作成が重要であり，現在のところ，このようなリンケージ分析に有効なマーカープローブ約700が分離されている．

B) 家系：家族数の多い3世代にわたる家系が望まれる．たとえば，米国のユタ大学では1世代当たりが8人以上の兄弟・姉妹よりなり，しかも両親・祖父母とも健在という家系を 46，フランスの CEPH (Centre d'Etude du Polymorphisme Humain) でも同様な家系13とユタ大学のものを含め，計59家系を用いてリンケージ分析を行っている．

C) リンケージ分析と染色体マッピング：リンケージ分析による家系分析は必ずしも染色体上のマッピングを意味するものではないが，用いたプローブの染色体上の位置づけをすることにより解決される．

このように DNA マーカーを用いたリンケージ分析からのヒト遺伝子のマッピングは未知の遺伝子を知る上で有効であり，Duchenne 型および Becker 型筋ジストロフィー，Huntington 病，cystic fibrosis，家族性大腸ポリポーシス，家族性アルツハイマー病など10数種の遺伝病の原因遺伝子の染色体上の座位が明らかにされ，その遺伝子の本態がしだいに明らかにされつつある．

HGM9 で明らかにされたマッピングされたヒト遺伝子数は約1,500である．また，各染色体上の DNA 断片が2,150ほど分離されていて，これらを合わせると，3,600を超える遺伝子，DNA 断片などが各染色体上に位置づけされている．