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ABSTRACT

Majority of the genes are evolving under the neutral mutation pressure. However, some genes are evolving through positive selection. Blood types were originally distinguished by the different molecular structure on erythrocytes. Therefore these products of blood group genes may cause interactions with other organisms, and there is possibility of positive selection on those genes. Because the Rh blood group gene products are membrane proteins, these products of blood group genes seemed to be affected by interactions with other organisms or cells on surface regions. It is known that the Rh blood group genes have homologous genes named Rh50, and hominoids have two or three Rh blood group genes. Therefore the Rh blood group genes and their related genes experienced a series of gene duplication events. Analyses of gene duplication events are also important to elucidate evolutionary rates and patterns of these genes. I thus analyzed the Rh blood group genes and their related genes from primates to fish to clarify the tempo and mode of evolution of these genes.

The human Rh blood type is one of the major blood group systems, and plays important roles in transfusion and clinical medicine, including haemolytic diseases of newborns, autoimmune diseases, and mild haemolytic anemia. Landsteiner and Wiener detected an antibody that agglutinates blood cells from rhesus macaques, and it was named Rh. Nucleotide sequences of Rh blood group genes in some primates were reported, and the phylogenetic relationship of primate Rh blood group genes have been conducted. However, the phylogenetic relationship of primate Rh blood group genes from these studies is not compatible with each other. Because hominoids have two or three loci of Rh blood group genes by gene duplication, gene conversion events (or some kind of convergent effects) may prevent to determine the true gene tree.

I examined the evolution of the Rh blood group genes of primates. Because we don't know the actual gene tree topology of primate Rh blood group genes, I assumed two plausible trees from nucleotide sequence data by using phylogenetic networks. I used the site by site reconstruction method under the maximum likelihood estimates to identify regions of gene conversion events assuming the two trees, and detected 9 or 11 converted regions. After eliminating the effect of gene conversions, I estimated numbers of nonsynonymous and synonymous substitutions for each branch of the both trees. Whichever we selected gene trees, the branch connecting hominoids and Old World monkeys

showed significantly higher nonsynonymous than synonymous substitutions, that is, indication of positive selection by using a statistical test. Many other branches also showed higher nonsynonymous than synonymous substitutions, and this suggests that the Rh genes have experienced some kind of positive selection. In any case, we should be very careful when we analyse the evolutionary history of tandemly duplicated genes, for there is always possibility of gene conversions.

To examine evolutionary patterns of other mammalian Rh blood group genes, I determined complete coding regions of Rh blood group genes of five mouse subspecies and rat, and Rh50 genes of five mouse subspecies, rat, and crab-eating macaque, and examined these genes. Nucleotide and amino acid sequence similarities between Rh genes and Rh50 genes are 47.2-48.9 % and 34.4-37.8 %, respectively. Comparison of synonymous and nonsynonymous substitutions for the Rh50 gene also revealed a possibility of existence of positive selection for this gene in primates. Because primates showed more clear sign of positive selection than rodents both for Rh and Rh50 genes, it is possible that the pattern of host-parasite interaction is different between primates and rodents. Phylogenetic analyses of Rh and Rh50 amino acid sequences indicate that the Rh50 gene has been evolving about two times more slowly than the Rh blood group gene both in primates and rodents. This conservative nature of the Rh50 gene suggests its relative importance to the Rh blood group gene. From the comparison of synonymous substitutions between Rh and Rh50 genes, it is suggested that the mutation rate of rodents is about three times higher than that of primates, and the divergence time between mouse and rat is estimated to be ca. 30 million years ago.

I also determined the Rh50-like genes of *Xenopus* and Japanese medaka and examined the long-term evolution of Rh, Rh50, and their related genes. The phylogenetic tree shows four clusters in this tree; Rh50 genes of mammals and the *Xenopus* Rh50-like gene, Rh genes of mammals, the Rh50-like gene of Japanese medaka, and two genes of *C. elegans*. Therefore, the *Xenopus* Rh50-like gene is probably orthologous to the Rh50 genes of mammals.

The topology of the phylogenetic tree suggests that the gene duplication of Rh and Rh50 genes occurred just before or after the divergence of teleost fish and other vertebrates. The branch lengths of Rh50 genes are much shorter than those of Rh genes, indicating a lower evolutionary rate in the Rh50 gene than in the Rh gene. Because its evolutionary rate is lower than that for the Rh protein gene, the Rh50 protein may be closer to the ancestral form before the gene duplication of

Rh and Rh50 genes. The time of gene duplication that produced the Rh and Rh50 genes was estimated to be about 450-480 million years ago. This period roughly corresponds to the early Paleozoic, around the divergence between tetrapods and teleost fish lineages.

From database searches, it is suggested that the Rh blood group genes and their related genes are related to ammonium transporter genes of many organisms, especially trans-membrane domains. The phylogenetic tree for ammonium transporter proteins indicated two major groups for ammonium transporter proteins. I propose to call these two groups of ammonium transporter genes as α and β groups, and the Rh genes group is more similar to the amt β group than to the amt α group. It is suggested that the Rh blood group genes and their related genes have probably been existing as essential membrane proteins in many animal phyla.

論文の審査結果の要旨

多くの遺伝子では、個体の生存にとって有害かあるいは中立な突然変異圧に曝されていることが知られている。しかし、いくつかの遺伝子では、生存に有利な突然変異も生じており、いわゆる「正の自然淘汰」が働くと考えられている。特に、ABO等の血液型遺伝子は、過去の集団遺伝学的研究から、正の自然淘汰を受ける遺伝子の有力候補として、分子レベルのより詳細な研究が待たれていた。このような状況のもとに、本学位論文申請者は、Rh血液型遺伝子とその相同なRh50遺伝子に注目して、分子進化学的観点から実験的研究とデータ解析を行った。

まず、申請者は、霊長類のRh血液型遺伝子の進化過程を調べた。利用可能な塩基配列データを用いたネットワーク解析により、これらの遺伝子には2通りの系統樹が共に可能であると仮定できることを明らかにした。そして、これらの系統樹に基づいて、最尤法を用いて一個一個の塩基サイトを丹念に調べ、遺伝子変換が起こった可能性のある領域を同定した。これらの領域を排除した上で、系統樹の各枝ごとに同義置換数及び非同義置換数を比較解析した結果、旧世界ザルと類人猿をつなぐ枝では、非同義置換数が同義置換数より有意に上回っていることを発見した。このことから、Rh遺伝子にはある種の正の自然淘汰が働いていることを初めて明らかにした。また、他のほ乳類のRh血液型遺伝子の進化パターンを調べるため、5つのマウス亜種とラットのRh血液型遺伝子、および同じ5つのマウス亜種とラット並びにカニクイザルのRh50遺伝子の全タンパク質翻訳領域の塩基配列を決定した。特に、Rh50遺伝子において、同義置換数と非同義置換数の比較解析を行い、Rh50遺伝子にも、霊長類において正の自然淘汰が働いている可能性が強いことを発見した。

さらに、Rh50遺伝子及びその関連遺伝子における長時間スケールの進化過程を解明するため、アフリカツメガエルと日本メダカのRh50様遺伝子の塩基配列を決定し、それらを含めた系統樹を構築した。その結果、アフリカツメガエルのRh50様遺伝子は、ほ乳類のRh50遺伝子と直系関係にあることを明らかにした。また、塩基置換速度の解析からRh遺伝子とRh50遺伝子の分岐時間はおよそ4億8千万年前であることも推定した。さらにまた、これらのRh遺伝子とアンモニウム・トランスポータ遺伝子との進化系統関係も明らかにした。

このような申請者のRh血液型遺伝子に関する研究は、この遺伝子とその相同遺伝子の進化過程を詳細に解析した初めての報告であり、正の自然淘汰を受けている遺伝子を探索する方法として、ネットワークを利用した遺伝子変換領域の排除など、ユニークな方法論を提示している。特に、この方法論は、この種の研究を今後行う上で、非常に有用と思われる。よって、本論文の内容は、申請者の研究能力を十分に反映しており、関係分野における貢献度も十分と考えられることから、学位を授与するに相応しいと判断された。