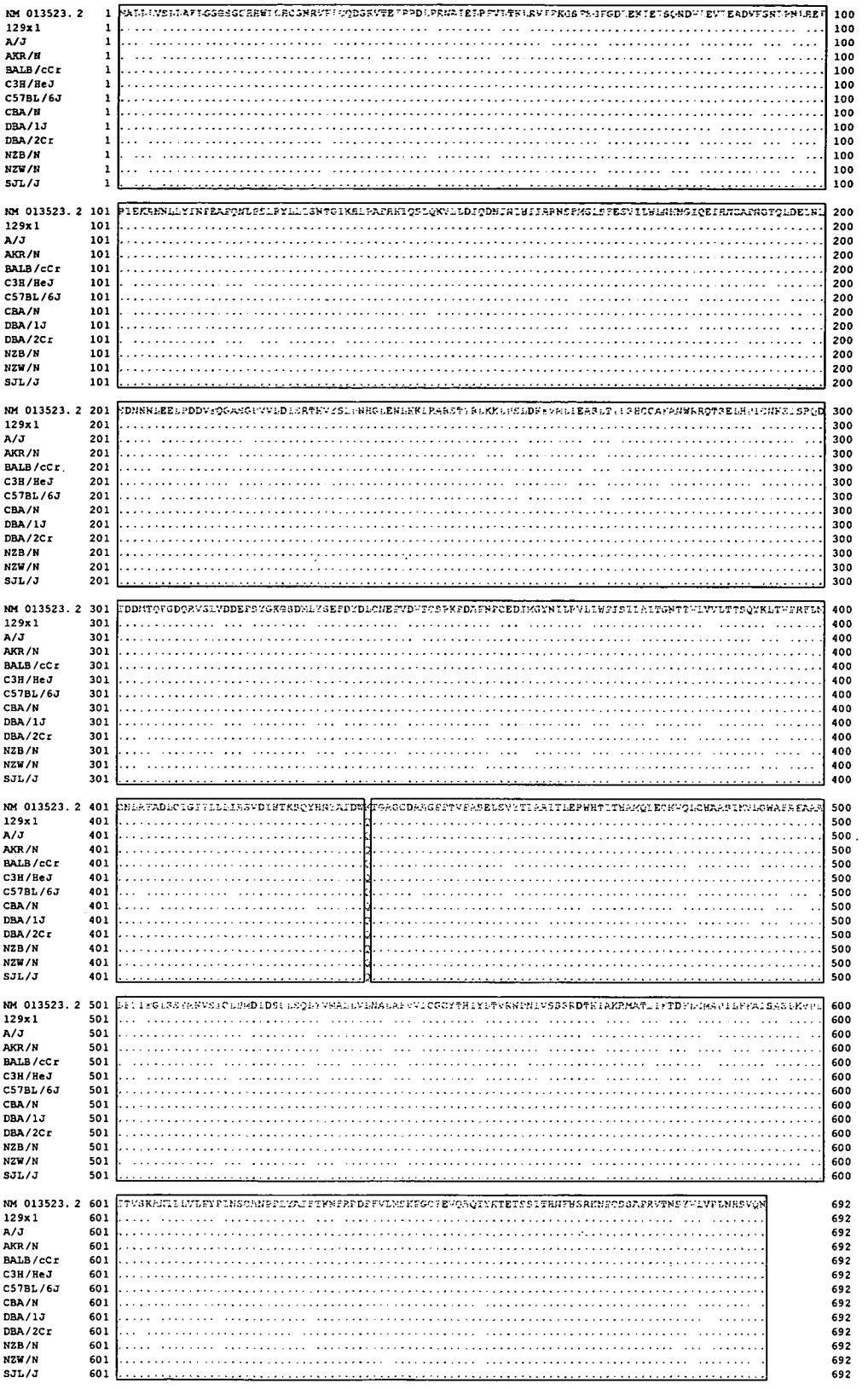


図6　12系統のFSHRのアミノ酸配列。GenBankの標準FSHR配列NM_013523.2より想定されるアミノ酸を基準にアラインメントを求めた。マウス12系統のFSH受容体アミノ酸配列には全く差異はないが，NM_013523.2とは1アミノ酸の差異があり，NM_013523.2が間違えている可能性がある。

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書

# マウス標準系統のプロファイリング

**分担研究者：内尾こずえ（独）医薬基盤研究所 生物資源研究部 研究員**

**研究要旨**

遺伝子改変マウス、すなわちトランスジェニック（TG），ノックアウト（KO）およびノックイン(KI)マウスの作成においては、対象とする系統マウスの遺伝的背景と発現型との関連が不明であり、作成した系統の表現型を予測することは非常に困難である。遺伝子改変マウスにおける改変遺伝子の発現型に関わる遺伝的要因と環境要因との関連を明らかにするため、遺伝的背景の異なる標準系統の遺伝的、生理的特性を融合したデータベースを整備することで遺伝子改変マウスの効率的な作成指標を提供し、効率化を目指す。

**A.研究目的**

遺伝子改変マウス作成法が広く普及したことで、マウス利用は増加の一途を辿っている。マウス標準系統として C57BL/6、BALB/c、DBA/2、C3H、FVB など様々な近交系マウスが利用されているが、マウスの遺伝的背景と発現型との関連が不明であり、作成した系統の表現型を予測することは非常に困難である。さらに食餌などの環境要因が表現型に及ぼす影響も大きく、疾患モデルマウス作出の際には考慮する必要がある。そこで多様な遺伝的背景と的背景と環境要因との相互作用に関わる遺伝的・生理学的情報をデータベース化し、モデルマウス作出法の効率化を目指す。

**B.研究方法**

＜供試動物＞

日本クレアより８週齢の C57BL/6J、BALB/cA および DBA/2J を購入した。マウス用飼料として通常食（CE2），低タンパク食（CE7）および高脂肪食（Quick Fat: QF）を用いた（日本クレアより購入)。実験スケジュールは図１、実験群は表１に示した。一晩絶食後、血液、臓器（肝・腎・膵・筋・白色脂肪・褐色脂肪）を採取した。

＜生化学検査＞

血清中のアルブミン、クレアチニン、グルコース、コレステロール、トリグリセライド（TG)、尿素窒素（BUN)、アミラーゼ、尿酸脱水素酵素（LDH）、alanine

aminotransferase （ALT）、aspartate aminotransferase (AST)、クレアチンホスホキナーゼ (CPK) を富士ドライケム 7000V にて測定した。また尿中のタンパク、クレアチニン、グルコースを測定した。

**C.研究結果**

今年度は生活習慣病のパラメーターを中心に測定を行った。

＜体重変化＞

飼料負荷後、週１回の体重測定を行ったところ、各系統オスマウスは高脂肪負荷により体重増加が認められた。特に DBA/2 オスマウスの体重増加が顕著であった。メスマウスは飼料の違いによる体重変化は観察されなかった。

＜肝機能　図２参照＞

ALT, AST 値を測定したところ、すべての C57BL/6 マウス実験群において低値であった。一方、DBA/2 オスマウスについては、高脂肪食群において、ALT, AST 値が増加した。今回、供試した３系統について、メスマウスの ALT, AST 値はオスマウスより低い値であり、食餌の影響をほとんど受けることはなかった。また LDH 値についても ALT, AST 値と同様に、C57BL/6 マウス実験群において低値であり、DBA/2 が高値であった。以上の結果より、C57BL/6 マウスの肝機能が優れていることが示唆された。

＜高脂血症パラメーター　図３参照＞

C57BL/6 マウス実験群においては、コレステロール値に性差や食餌の影響はほとんどなかった。一方、DBA/2 および BALB/cA マウスについては、メスよりオスのコレステロール値の方が高く、雌雄差が明確であった。また DBA/2 が食餌の影響を受けやすい傾向にあった。トリグリセライド (TG) については、DBA/2 マウスはメスよりオスの TG 値の方が高く、雌雄とも高脂肪食により TG 値が増加したが、C57BL/6 および BALB/cA 群は、食餌の影響や雌雄差による顕著な変化は認められなかった。以上の結果より、DBA/2 オスマウスは高脂血症感受性が高く、C57BL/6 は抵抗性の傾向にあることが示唆された。

＜筋　図４参照＞

CPK を測定したところ、すべての C57BL/6 マウス実験群において低値であった。一方、DBA/2 オスマウスは高脂肪食摂取により、顕著に増加する傾向にあった。また各系統メスマウスは食餌の影響をほとんど受けず、低い値であった。

現在、血清中のインスリン、アディポネクチン、レプチン、TNFαや IL6 等の各種サイトカイン値を測定するとともに、各臓器の遺伝子発現を解析中である。

**D.考察**

本研究で用いた 3 系統において、オスマウスが食餌の影響を受けやすく、生活習慣病になりやすい傾向が示唆された。また C57BL/6 マウスは生活習慣病抵抗性の傾向が強く、DBA/2 マウスは感受性が高いことが示唆された。これらの結果からも遺伝子改変マウス作成の際に各疾患にあった系統を選択する重要性が分かる。一般的に C57BL/6 マウスが遺伝子改変マウス作成に用いられているが、糖尿病などの生活習慣病研究を目的とする場合は、他系統の利用も視野に入れる必要がある。今後、血清中のインスリン、アディポネクチン、レプチン、TNFαや IL6 等の各種サイトカイン値のデータを集積するとともに各臓器の遺伝子発現の解析を続ける所存である。さらに FVB, C3H, A/J 等のマウス系統についても同様の検討を進め、各種疾患モデルマウス作成支援データベースを構築していきたい。

**E.結論**

食餌などの環境要因が表現型に及ぼす影響は、マウス系統ならびに性別によって異なる。このことは遺伝的要因と環境要因には関連性があることを示唆している。遺伝子改変マウス作出の際には、最適な系統を選択し、効率化を図ることが望ましい。本研究によるマウス系統遺伝的・生理学的情報データベース構築により、モデルマウス作成支援に貢献していきたい。

**G.研究発表**

1. 論文発表

なし

2. 学会発表

1. 山田-内尾こずえ・澤田京子・國枝孝典：ネフローゼマウス(ICGN マウス)の病態進行パラメーターについて、第 144 回日本獣医学会、2007 年 9 月 2-4 日、江別
2. 國枝孝典・澤田京子・内尾こずえ：マウス卵胞発育過程における TNFαおよび TNF 受容体の発現解析、第 100 回日本繁殖生物学会、2007 年 10 月 18-22 日、東京
3. 山田-内尾こずえ・澤田京子・眞鍋昇：Matrix Metalloproteinase-12 はネフローゼマウス(ICGN マウス)の病態進行に関与する、第 145 回獣医学会、2008 年 3 月 28-30 日、相模原

図１

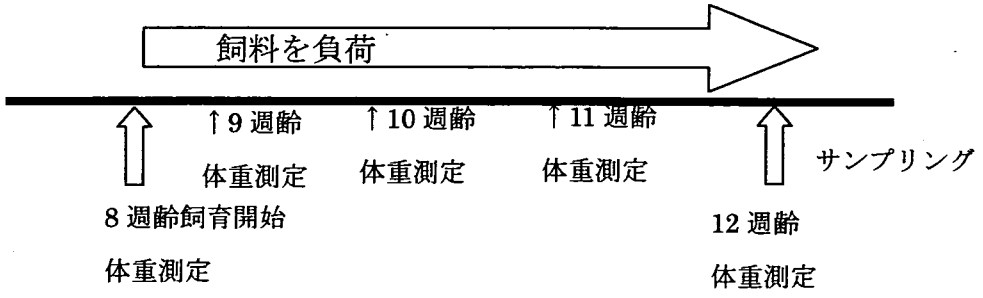



表１

| 実験群 | 系統名 | 雌雄 | 飼料 | 実験群 | 系統名 | 雌雄 | 飼料 |
|---|---|---|---|---|---|---|---|
| 1 | C57BL/6J | オス | CE2 | 10 | C57BL/6J | メス | CE2 |
| 2 | C57BL/6J | オス | CE7 | 11 | C57BL/6J | メス | CE7 |
| 3 | C57BL/6J | オス | QF | 12 | C57BL/6J | メス | QF |
| 4 | DBA/2J | オス | CE2 | 13 | DBA/2J | メス | CE2 |
| 5 | DBA/2J | オス | CE7 | 14 | DBA/2J | メス | CE7 |
| 6 | DBA/2J | オス | QF | 15 | DBA/2J | メス | QF |
| 7 | BALB/cA | オス | CE2 | 16 | BALB/cA | メス | CE2 |
| 8 | BALB/cA | オス | CE7 | 17 | BALB/cA | メス | CE7 |
| 9 | BALB/cA | オス | QF | 18 | BALB/cA | メス | QF |

図２

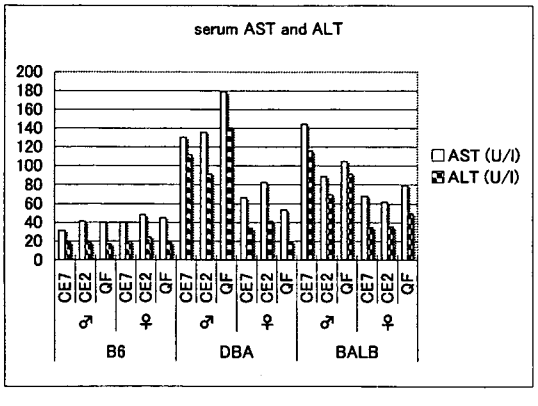



図３

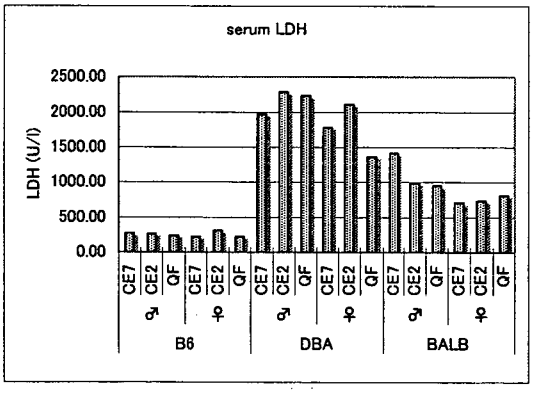


図4

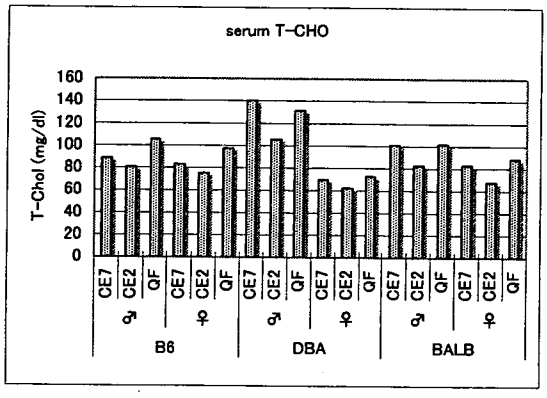
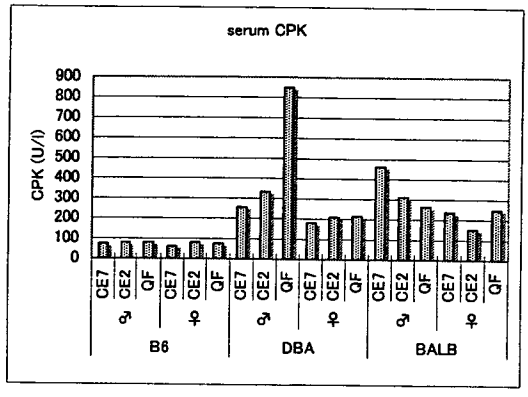

serum T-CHO
T-Chol (mg/dl)
160
140
120
100
80
60
40
20
0
CE7
CE2
QF
♂
♀
B6
DBA
BALB

図5


serum CPK
CPK (U/l)
900
800
700
600
500
400
300
200
100
0
CE7
CE2
QF
♂
♀
B6
DBA
BALB

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書

# カニクイザル cDNA クローンの収集解析と ゲノムマーカーの開発整備

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**分担研究者　高橋一朗（医薬基盤研究所・生物資源研究部・遺伝子資源室）**

**研究要旨**

カニクイザルの骨髄，脾臓の各組織からcDNAライブラリーを作成し、各臓器から10，000クローンのタグシークエンス解析を行い新規cDNAクローン約150個を当室のcDNAコレクションに加えた。またゲノムマーカー開発整備として384種のヒトマイクロサテライトマーカープローブによりヒトーカニクイザル間で保存的でかつ多型を示す座位106箇所を検証した。

カニクイザルは医薬品の効果や安全性また疾患感受性解析などの医学実験に最も頻繁に用いられている霊長類研究資源であり、その遺伝的背景を明確にすることは医学研究推進にとって重要である。我々はこれまでにヒトホモログとしてのカニクイザルcDNAを10754クローンを収集してきた。RefSeqに登録されているヒトの標準遺伝子は約2万4千個であり、カニクイザルではさらなるヒトホモログcDNAの収集が必要である。今年度は骨髄及び脾臓のcDNAライブラリー各10000クローンのタグシークエンス解析から約150個の新規cDNAクローンを収集した。また、カニクイザルにおいては疾患感受性遺伝子座や薬剤感受性遺伝子の解析に欠かせないゲノムマーカーも不足している現状から、ヒトとの保存的座位を利用した106個のクロサテライトマーカープローブが利用できることを明らかにした。

## A. 研究目的

カニクイザルはその近縁種のアカゲザルとともに最も頻繁に医学実験に用いられている霊長類のひとつである．アメリカではその医学的重要性を理由にアカゲザルの全ゲノム配列解読が行われ，2007年にその概要版が発表された．しかし，それに対してカニクイザルのゲノム情報は圧倒的に少ない状況である．また，アカゲザルのゲノム配列のどの部分が実際に転写されているかについての情報も非常に少ないのが現状である．本研究ではカニクイザルの遺伝子情報であるcDNAを単離してその配列を解析することにより，カニクイザルの遺伝情報をより高密度にし，将来のサルを使った遺伝的解析の基盤的整備を行うことを目的とする．また，アカゲザルとカニクイザルのゲノムレベルおよび転写産物レベルでの近縁性を探ることにより，アカゲザルのゲノム情報がカニクイザルの研究にどれだけ有用であるかについて検討を行う．また、カニクイザルの遺伝的解析においては連鎖解析や繁殖コロニー、家系間の解析に用いるゲノムマーカーの整備が遅れていることから、ゲノムマーカーの整備が進んでいる既存のヒトマイクロサテライトマーカープローブを用いて、ヒトーカニクイザル間で座位特異的配列が保存的でかつ多型性を示すプローブの選抜を行うことにより、カニクイザルに適用できるゲノムマーカープローブの整備に貢献することを目的とした。

**B. 研究方法**

カニクイザルの遺伝情報の比較にはこれまで解読された約 10,000 のカニクイザル cDNA 配列(公共データベースに登録済み)およびヒト, アカゲザルのゲノム配列を用いた. 新規 cDNA については, 骨髄, 脾臓の各組織からオリゴキャッピング法により完全長 cDNA ライブラリーを作成した(東大菅野研究室との共同研究). ライブラリー作成に用いられたサルサンプルの採取方法については医薬基盤研究所動物実験倫理指針に基づき, 倫理審査委員会にて承認された. ゲノムマーカープローブの整備については、フィリピン由来 16 頭、インドネシア由来 14 頭、マレーシア由来 14 頭よりゲノム DNA を得て、ヒトマイクロサテライトマーカープローブ HD25 および HD5(ABI 社)の 384 種を用いて、カニクイザル個体ゲノム DNA をテンプレートとした PCR 産物を GeneMapper を用いて鎖長解析と多型の解析を行った。

**C. 研究結果および考察**

カニクイザル骨髄, 脾臓からそれぞれ 10,000 ずつの cDNA クローンを単離し, ABI3730 シークエンサーをもちいてそれぞれ約 9,000 および約 5,000 のクローン配列を決定した. 解読された配列はヒト遺伝子との相同性により注釈づけられ, カニクイザル cDNA データベース(QFbase, http://genebank.nibio.go.jp/qfbase/)に登録されている. これらのデータは遺伝子機能などにより容易に検索が可能である. また, これまでに解読されたカニクイザル cDNA のなかから, ヒトで見つかっていない新規転写産物についての解析をカニクイザルオリゴ DNA アレイを用いて行った. その結果, これらの未知転写産物の約半数は非常に弱いレベルで, しかし有意なレベルで発現されていることがわかった. その多くは組織特異的な発現パターンを示した. さらに, カニクイザル cDNA 配列とアカゲザルゲノム概要配列を比較することによって, 両者の平均的な遺伝距離が約 0.4-0.5%であることが判明した. この結果はカニクイザルとアカゲザルが非常に近縁で, 遺伝子情報がお互いにかなりの部分共有できることを示している.

カニクイザルゲノムマーカー整備としてヒトマイクロサテライトマーカープローブ(HD25、HD5、ABI 社)セットよりヒト常染色体上のプローブ 384 個についてカニクイザルーヒト間での保存性とについて検証し106個の座位が保存的でありマイクロサテライトを検出した。106 個の内 72 個については由来地間、個体間で多型性を示しておりゲノムマーカーとして利用できることを確認した。残り 36 個についてはマイクロサテライト配列は検出するが多型性に関して検証個体を増やすなどして検討する必要がある。確実に検出できる 70 個のマーカーを表1に示した。フィリピン、インドネシア、マレーシアの各由来地特異的に検出できたマカーもあり、このようなプローブも繁殖群特異的な解析に有効に利用できることが考えられた。

(考察)

現在までの努力の結果, 全ヒト標準遺伝子のうちおよそ半数の遺伝子についてサル cDNA を得ることができた. このデータはカニクイザルを使った研究のみならず, アカゲザルを用いた研究にも利用することができる. 特にアカゲザルではゲノム配列は決定されたが転写産物に関する情報が少ないために, 遺伝子の詳細な注釈が不可能であった. 我々のデータはこれを補完し, 霊長類を用いた医学実験の向上に資することが期待できる。カニクイザルゲノムマーカーの整備は疾患モデルや薬剤感受性などの遺伝的背景探索や関連遺伝子探索に必要不可欠なものである。カニクイザルゲノム配列データベースはまだ存在せず、アカゲザルゲノム情報を基にカニクイザルのゲノムを推測するのが現状である。カニクイザルゲノム情報を基に、今回利用可能であることが検証されたプローブの内1番染色体上のプローブについて仮想的にカニクイザル1番染色体上にマップした(図1)。カニクイザルゲノム上の正確な位置については当該するゲノム部位の BAC クローン解析などを進めて行く必要がある。

**E. 結論**

本研究により，カニクイザルの cDNA 配列が蓄積され，世界最大規模の cDNA データベースの充実を図ることができた．また，アカゲザルとの比較により，この遺伝情報はカニクイザル研究のみならず，アカゲザルやニホンザルを用いた研究にも非常に有用であることが判明した．近年，サル類を用いた研究のなかで遺伝情報はより重要な役割を担うことになり，ますます整備されていかなければならないと考えられる．そのために今後も様々な組織から遺伝子クローンを単離してコレクションを拡充していくことが必要である。

**F. 健康危険情報**

特になし

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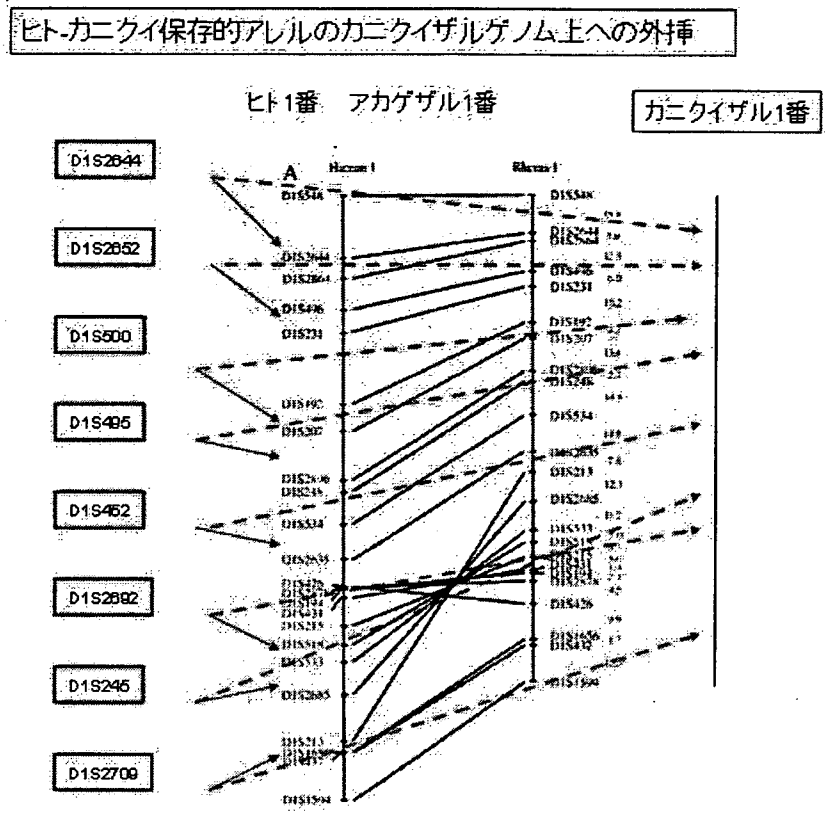
**H. 知的所有権の出願・登録状況**

なし

表1
ヒトーカニクイザル間で保存的かつ多型性のマイクロサテライト

| | | | |
|---|---|---|---|
| D1S2644 | D2S2361 | D6S281 | D7S2560 |
| D1S500 | D3S1600 | D6S262 | D7S2252 |
| D1S2652 | D3S3592 | D6S434 | D7S2496 |
| D1S2692 | D3S1555 | D6S462 | D8S549 |
| D1S245 | D3S1609 | D6S1610 | D8S258 |
| D1S2709 | D3S3634 | D6S1609 | D8S270 |
| D1S2766 | D3S3521 | D6S1660 | D12S86 |
| D1S452 | D3S3668 | D6S264 | D12S336 |
| D1S495 | D4S2971 | D6S276 | D18S68 |
| D1S500 | D4S2994 | D6S1697 | D20S889 |
| D1S233 | D4S1595 | D6S470 | D20S117 |
| D2S118 | D4S3022 | D6S1575 | D20S115 |
| D2S2202 | D5S495 | D6S291 | |
| D2S163 | D5S406 | D6S270 | |
| D2S306 | D5S422 | D6S1721 | |
| D2S362 | D5S1981 | D6S259 | |
| D2S2264 | D5S1969 | D6S1599 | |
| D2S388 | D5S471 | D7S530 | |
| D2S2369 | D5S2050 | D7S691 | |
| D2S2344 | D5S2084 | D7S2423 | |

図1


ヒト-カニクイ保存的アレルのカニクイザルゲノム上への外挿
ヒト1番　アカゲザル1番
カニクイザル1番
D1S2644
D1S2652
D1S500
D1S495
D1S452
D1S2692
D1S245
D1S2709
A

# 研究成果の刊行に関する一覧表

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# Evolution of Gene Expression in Human and Chimpanzee Brains

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Advanced article

Article Contents



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The deoxyribonucleic acid (DNA) microarray technique enables us to gauge the difference in gene expression level between human and chimpanzee brains at a genome-wide level. Several studies have shown that (1) gene expression is more conservative in the brain than in other tissues, (2) the divergence rate of gene expression in the brain is higher in the lineage of humans than in the chimpanzees and (3) more genes are up-regulated in human brains.

s0001

## Introduction

p0001 The brain is one of the most fascinating and biologically prominent organs of human beings. What is the genetic basis of the higher cognitive ability of humans, which bestowed on us the use of fire, sophisticated languages, arts and an inquisitive mind that searches the roots of humanity itself? After almost 150 years after the first insights of Charles Darwin, recent studies of molecular biology have presented unquestionable evidence that our closest relatives are chimpanzees and bonobos. These studies have accumulated a large amount of molecular data that reveal the phylogenetic relationship of higher primates (**Figure 1**) and show that the genetic difference between humans and chimpanzees is surprisingly small. Now that the genomes of the humans and chimpanzees have been sequenced, we know that only approximately 1–2% of their genomes differ at the deoxyribonucleic acid (DNA) sequence level AU:1 (Chimpanzee Sequencing and Analysis Consortium, 2005). The amino acid sequences of structural proteins are usually more similar than the DNA sequences, because not all changes in the DNA sequence affect the protein-coding sequence and many of the possible changes in protein sequences could be deleterious to the organisms. The difference between humans and chimpanzees at the amino acid sequence level is only about 0.2%. These observations suggest that the differences in gene expression proteins are more important than those in structural proteins for determining the phenotypic differences between humans and chimpanzees (King and Wilson, 1975). **See also**: Great Apes and Humans: Genetic Differences AU:2

p0002 The most striking form of difference in gene expression would be gene deletion, which leads to the complete absence of expression of the deleted gene. Similarly, gene duplication may magnify the gene expression of the duplicated genes; however, other quantitative differences in gene expression may have occurred because of *cis*- and *trans*-regulatory mutations in the lineage of humans and/or chimpanzees. *Cis*-regulatory mutations are mutations that reside at a site adjacent to a gene and directly alter its expression, such as mutations in promoter sequences; and *trans*-regulatory mutations reside elsewhere in the genome. If *cis*-regulatory mutations change the expression of genes that affect the expression of other genes (e.g. transcription factor genes), the *cis*-regulatory mutations could be, in turn, *trans*-regulatory mutations with respect to other genes. These mutations can modify the gene expression pattern of many genes expressed in the brain and may change the phenotype of the brain drastically. **See also**: Gene Duplication: Evolution; Primate Evolution: Gene Loss and Inactivation

p0003 The original hypothesis of King and Wilson had to wait a long time before it was underpinned by experimental data. The advent of DNA microarray technology has enabled us to measure the difference in gene expression between humans and chimpanzees at the genome-wide level. In DNA micorarray experiments, messenger ribonucleic acids (mRNAs) are reverse-transcribed to complementary DNAs (cDNAs), labelled and hybridized to the oligo-DNAs or cDNAs that are arrayed densely on a glass surface. The number of mRNA molecules in a sample can be measured from the relative signal intensity of each probe. Since DNA microarrays can detect expression of thousands of genes, many studies have used microarrays to

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explore the differences in gene expression between human and chimpanzee brains at the genome-wide level. **See also:** DNA Chips and Microarrays

p0004 The first genome-wide comparison of gene expression in human and chimpanzee brains was performed by Enard *et al.* (2002). Since then, several studies have reanalysed their data or performed analyses using different materials and methods. Although there are many technical problems in the use of DNA microarrays and some results have been contradictory, there is a thread of experiments that offers a general picture on how the brains of humans and chimpanzees differ in gene expression. Several new approaches, using methods such as network analysis, genome tiling arrays and massively parallel sequencing offer novel insights into how this difference is reflected at many different levels of transcriptome regulation.

## s0002 Comparison of Expression Level Using DNA Microarrays

### s0003 Evolutionary conserved gene expression pattern in the brains

p0005 The initial analysis by Enard *et al.* (2002) indicated that the acceleration of gene expression changes in the lineage of humans relative to chimpanzees is greater in the brain than in the liver, conveying false impression that total amount of changes in gene expression is greater in the brain than in the liver. Subsequent reanalyses, however, revealed that their data indicate smaller number of differentially expressed genes in the brain than elsewhere (Cáceres *et al.*, 2003; Gu and Gu, 2003). In fact, among the examined thus far, such as the liver, heart and testis, the brain has the most conservative pattern of gene expression in humans and chimpanzees (Cáceres *et al.*, 2003; Khaitovich *et al.*, 2005). **Figure 2** shows the number of genes showing a difference in expression humans and chimpanzees determined by Khaitovich *et al.* (2005) using Affymetrix U133plus2 arrays. The figure shows that the divergence in the evolution of gene expression between humans and chimpanzees is least pronounced in the brain. In other words, the brain is an unusual organ where the global pattern of gene expression is highly conserved between humans and chimpanzees. This observation implies that the functional constraint on gene expression is stronger in the brain than elsewhere; i.e. changes in expression tend to be deleterious more often in the brain than in other tissues.

p0006 Despite of the earlier misunderstanding, the study by Enard *et al.* (2002) provides important findings that underpin the hypothesis of King and Wilson, because of the emphasis laid on the unequal distribution of evolutionary changes between the human and chimpanzee lineages, which will be discussed in the next section.

### Lineage imbalance of changes in gene expression in the brains s0004

Although the difference in gene expression is relatively small in the brain, using outgroup information, we can infer which lineage – humans or chimpanzees, after their split from the common ancestor – accumulated more changes in gene expression than the other. Orangutans and Old World monkeys are reasonable choices for the outgroup species. If the gene expression level is similar for chimpanzees and the outgroup species but significantly different in humans, one can infer that the difference occurred in the human lineage after the spilt from the chimpanzee lineage. By assigning a direction for changes in gene expression, an excess of expression divergence in the human lineage relative to the chimpanzee lineage was observed only in the brain, not in other tissues (Gu and Gu, 2003; Cáceres *et al.*, 2003). **Figure 3** shows the number of differentially expressed genes in the human and chimpanzee lineages, using orangutans as the outgroup. The data were the reanalysed data of Enard *et al.* (Gu and Gu, 2003). As mentioned in the previous section, the total number of differentially expressed genes is greater in the liver than in the brain. However, the number of changes in the human lineage relative to those in the chimpanzee lineage is significantly greater in the brain than in the liver, indicating that more gene expression changes have accumulated in the human brain after the split from the common ancestor. p0007

These observations suggest that the small changes in expression in the brain reflect strong functional constraint on the brain and even the smallest excess of human-specific changes correlates with the phenotypic advancement of the human brain. p0008

### Excess of up-regulated genes in the human brain s0005

The studies described earlier have also found that there was more upregulation than downregulation of brain-expressed genes in the human lineage. This imbalance has been found only in genes expressed in the human brain. For example, Cáceres *et al.*, using several methods, identified 91 genes that showed high or low expression only in the human cortex compared with the cortex of the chimpanzee and macaque. Among these 91 genes, 83 were up-regulated in the human cortex, whereas only 8 were down-regulated. The increased expression of these genes could be related to the prominent neuronal activity of the human brain, and these genes are therefore interesting candidates for detailed studies of their biological function. p0009

### Gene network differences between human and chimpanzee brains s0006

Instead of pursuing the list of differentially expressed genes, a more systematic approach was used in a recent study (Oldham *et al.*, 2006). The phenotypic differences in the brains may be a product of an interacting gene network p0010

rather than the additive effects of individual gene functions. Therefore, it has been hypothesized that the gene interaction network in the human brain would be more complex than the network in the chimpanzee brains. Microarray experiments produce richer information than a mere list of differentially expressed genes. Oldham *et al.*, performed a network analysis of expression data across six brain regions in 18 humans and 18 chimpanzees. They identified several coexpressed gene network modules in the brains. Interestingly, the network connectivity of the cerebral cortex module showed the weakest conservation between humans and chimpanzees. **Figure 4a** shows an example of the visualization of a coexpressed network in the brain. In **Figure 4b**, only the connections that are present in humans, but absent in chimpanzees, are displayed. These network changes in the human cerebral cortex might be associated with the expansion of the region and responsible for the greater cognitive ability of humans.

s0007 ## Technical and theoretical problems with microarray experiments

s0008 ### Sequence mismatches

p0011 Although many empirical and statistical methods have improved the accuracy and reproducibility of DNA microarray experiments, there are technical problems with comparative DNA microarray studies using different organisms. Many studies used Affymetrix oligonucleotide microarrays that were using 25-mer oligonucleotide probes based on human genome sequences. The sequence difference in nonhuman primates, however, would result erroneously in weaker expression signals in the nonhuman primates because the signal detection of microarrays is affected by the sequence mismatches of DNA probes. In addition, sequence polymorphisms within the species might distort the hybridization pattern. Now that the draft genome sequences of the chimpanzee and macaque have are available, this problem can be partially fixed by eliminating probes whose sequences are different from those in the human genome. Alternatively, the signal intensity of genomic DNA hybridization can be used to calibrate sequence mismatches, because DNA of two species that hybridize to the probes are supposed to be equal in amount. Microarrays carrying longer probes, such as cDNA microarrays, are thought to be less affected by sequence mismatches.

s0009 ### Choice of outgroup species

p0012 Gorillas, although are more closely related to humans and chimpanzees, are not an appropriate outgroup, because a considerable amount of the human genome is closer to the gorilla genome than to the chimpanzee genome owing to the manner of segregation of the ancestral polymorphisms. Although orangutans may be the ideal outgroup, the tissue samples from orangutans have been less readily than samples from more distant species, such as Old World monkeys, which are used as well-controlled experimental animals in biomedical research. **See also**: Great Apes and Humans: Genetic Differences

p0013 Information on outgroup is not always available because of various reasons. Without using the outgroup information, a recent study estimated the amount of changes in gene expression in the human and chimpanzee lineages, assuming that the upregulation of gene expression is of greater amplitude, but less frequent, than downregulation (Khaitovich *et al.*, 2005). They concluded that the genes expressed in the brain have changed their expression more in the lineage of humans than in the chimpanzees. If the assumption made by these authors is true, the findings that (a) more gene expression changes have occurred in the lineage of humans than in the chimpanzees and (b) more genes are up-regulated in the human lineage describe intrinsically the same biological phenomenon – if more gene expression changes have occurred in the human lineage, these expression changes must have resulted in the excess of upregulation under the model.

### Biological resources of primates s0010

p0014 Another problem is related to the availability of the biological resources of humans and other primates. As mRNA is a very fragile molecule and degrades quickly, it is ideal to sample tissues immediately after the individual has died. In case of humans, however, brain samples are usually obtained during autopsy, and the quality of samples for factors, such as age, sex and the post-mortem intervals is extremely difficult to control. The difficulty of obtaining suitable samples is similar or even greater for other great apes (chimpanzees, gorillas and orangutans) because of ethical issues and the small number of these animals in captivity.

### Some caveats from population genetics theory s0011

p0015 The earlier observations indicate the acceleration of gene expression in the brain only in the human lineage. Nevertheless, this observation might be explained by the fact that, historically, humans have had a much smaller effective population size than chimpanzees. According to the theory of population genetics, natural selection acts more weakly in smaller populations and more strongly in larger populations. Under the strict neutral theory of molecular evolution, the rate of molecular evolution does not depend on population size; however, under the nearly neutral theory, which permits the existence of slightly deleterious mutations, the molecular evolution of functional sites is predicted to be faster in small populations. What can population genetics tell us about the evolution of gene expression? Suppose that any changes in gene expression, changes in the brain are deleterious or slightly deleterious. In such cases, stabilizing selection can act on the pattern of gene expression, any individual that has unusual pattern of gene expression will be removed by natural selection. The intensity of natural selection has been weaker in the human population than in the chimpanzee population for a long period of evolution. In this situation, rapid evolution of

gene expression in the human brain might occur without any functional mechanisms. Therefore, it is important to note that the rapid evolution of gene expression in the human lineage has been observed only or most intensively in the brain and the conditions are satisfied by the series of microarray experiments. **See also**: Human and Chimpanzee Nucleotide Diversity; Natural Selection: Introduction; Population Genetics of Modern Human Evolution

## s0012 *Cis*-regulatory Mutations That Cause the Evolution of Gene Expression

p0016 What are the underlying mutations that cause the difference in gene expression between humans and chimpanzees? Previous studies have identified *cis*-regulatory human-specific mutations in transcription factor-binding sites (Donaldson and Göttgens, 2006), or promoter regions (Haygood *et al.*, 2007) and conserved noncoding elements (Prabhakar *et al.*, 2006). Interestingly, these studies found associations of human-specific mutations with genes categorized as having neuronal functions. Some of them might be the source of the human-specific evolution of gene expression in the brain; however, the biological significance of these regulatory mutations evolution of gene expression in the brain remains unclear, because the correlation between the regulatory mutations and gene expression differences is not very strong (Haygood *et al.*, 2007) and the *cis* effect is hard to verify using *in vitro* experiments (Heissig *et al.*, 2005).

## s0013 Gene Expression and Protein Sequence Evolution

p0017 Although the rapid gene expression evolution in the human brain is an attractive finding, the protein sequences themselves may be important in the phenotypic evolution of the brain. Recent studies have found that some genes that are related to brain function evolved rapidly under positive
AU:3 Darwinian selection, such as *FOXP2* (forkhead box P2), *ASPM* (abnormal spindle homologue, microcephaly associated) and *MCPH1* (microcephalin 1) (reviewed by Sik-
AU:4 era, 2006). Dorus *et al.* (2004) reported that a set of hundreds of genes related to the nervous system have evolved more rapidly in the lineage of humans than in the macaques. This finding suggests that some neuronal genes have evolved under positive selection and that protein sequences and gene expression are evolutionary coupled. A parallel pattern of protein sequence and evolution of gene expression has also been observed (Khaitovich *et al.*, 2005); i.e. if the amino acid sequence of a protein has evolved rapidly between humans and chimpanzees, the gene expression may also shows a large evolutionary difference in the tissue in which the gene is expressed.

p0018 On analysing brain-expressed genes more broadly, however, the opposite trend has been observed by other researchers, who concluded that brain-expressed genes evolved more slowly in the human lineage than those in chimpanzees (Wang *et al.*, 2007). Because any set of genes may have evolved more rapidly in the human lineage than in chimpanzees owing to the smaller population size of humans, to find out the relative rate of evolution in brain-expressed genes, it is necessary to calibrate the rate with respect to the genome-wide average. The results show that, although some functionally important genes have evolved rapidly because of positive selection, most brain-expressed genes have evolved rather slowly in the human lineage. This has been explained by the hypothesis that, in tissues that have a more complex gene network, functional constraints on genes are stronger than in tissues that have a simpler gene network. As a result, protein sequences of brain-expressed genes in humans have evolved at an extremely slow rate (Wang *et al.*, 2007). A similar trend has also been reported by Shi *et al.* (2006).

## Evolution of Noncoding RNAs s0014

### MicroRNAs s0015

Recently, the potential of *trans*-regulatory factors of large p0019 effect, such as microRNA (miRNA), has been discussed in detail. miRNAs are RNA molecules of approximately 22 nt that are processed from larger hairpin precursors. Most AU:5 miRNA sequences are highly conserved throughout evolution. Nevertheless, since a single type of miRNA can change the expression of hundreds of downstream genes, the difference in miRNA expression between human and chimpanzee brains might be a key to their large phenotypic differences. Berezikov *et al.* (2006) applied massively par- AU:6 allel sequencing of miRNAs in human and chimpanzee brains. After sequencing approximately 400 000 clones, they found many novel miRNA clones. They identified 76 miRNAs that were cloned only in one species and were not conserved in the other species. Until date, several novel techniques have been developed to enable DNA sequencing that may be faster by at least hundred times than the conventionally used Sanger sequencing method. Although the analysis of Berezikov *et al.* is rather preliminary, the new approaches may finally provide us with methods suited to the deep analysis of transcriptome evolution.

### Unknown intergenic transcripts s0016

As a result of large-scale cDNA sequencing efforts and p0020 studies using genome tiling microarrays, which array entire human genome sequences with a resolution of several hundreds of base pairs, we now recognize that a substantial part of the human genome is transcribed as noncoding RNA. The function of noncoding RNAs, however, remains unclear. If noncoding RNAs are mapped outside any known protein-coding genes, the transcripts are considered to be intergenic transcripts. Using human genome tiling arrays, Khaitovich *et al.* compared the expression

patterns of known genes and intergenic transcripts between humans and chimpanzees in several tissues (Khaitovich *et al.*, 2006). Overall, the divergence of expression was greater for intergenic transcripts than for known genes; however, the authors observed a parallel evolution pattern of expression between the known genes and the intergenic transcripts. The expression of intergenic transcripts was more conserved in the brain than in other tissues, such as heart, testis and lymphoblastoid cell lines, between humans and chimpanzees. This indicates that the functional constraint on brain-expressed transcripts is strong irrespective of whether the transcripts encode proteins. There are two possible explanations: the intergenic transcripts are actually functional in the brain by binding to mRNA of other genes or directly interacting with proteins; or the mutated intergenic transcripts cause interference with the other transcripts in the brain.

s0017 ## Conclusion

p0021 The establishment of the genome sequences of humans and chimpanzees was a milestone in our efforts to understand the origin of humanity. Using the two-genome sequences, we were able to identify differences between them at the DNA and amino acid sequence level; however, a limited amount of information can be obtained from the genome sequences themselves because the nature of the transcriptome (the amount and tissue distribution of the transcripts) cannot be directly predicted solely from the genome sequences. DNA microarrays enable us to make genome-wide comparisons of gene expression patterns between humans and chimpanzees. Several studies have found that (1) gene expression is more conservative in the brain than in other tissues, (2) the rate of divergence of gene expression in the brain is greater in the lineage of humans than in the chimpanzees and (3) more genes have been up-regulated in the brains of humans. The last two of these features have not been observed in other tissues, or these phenomena have been more pronounced in the brain than in other tissues.

p0022 The next step will be to identify the underlying mutations causing the evolution of gene expression and unveil how the genes involved make human brains through developmental processes and control human cognitive ability. Of course, our understanding of the transcriptome world is inadequate at this point in time. Recent studies have shown a potentially large effect of noncoding RNAs on the regulation of gene expression regulation. Moreover, we have little knowledge about the genetic basis of the mechanisms of brain functions. Future studies using novel approaches will further clarify how differences in gene expression make our brains different from those of chimpanzees.

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