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社団法人畜産技術協会附属
動物遺伝研究所

動物遺伝研究所年報　第十四号（平成十八年度）

社団法人畜産技術協会附属動物遺伝研究所

序　　文

本年報は平成18年度における附属動物遺伝研究所の研究の概要などを中心に、研究所の諸活動についてとりまとめたものです。

これまで研究所は、ウシゲノム解析ツールの開発を独自に担いつつ、黒毛和種牛を主たる研究対象として、DNA育種手法の実用化を目的とした研究を進めてきました。その成果の一つとして、DNAマーカーとの遺伝的な連鎖関係からいくつかの遺伝性疾病の原因遺伝子を同定し、不良因子を見分けるDNA診断法を開発することで不良因子キャリアのコントロールを可能にしてきました。しかしながら、クローディン-16欠損症ほどの影響力はないものの、依然として数々の不良因子が黒毛和種集団に残存していることから、今後とも育種現場との連携を密にして対処していかなくてはなりません。

また、経済形質責任遺伝子を同定することは夢物語ではない段階まで到達しました。当研究所を始めとする諸機関の努力によってウシゲノムツールが充実し、それを基盤に国際コンソーシアムがウシゲノム解読を順調に進めた結果、ヒト・マウスゲノム情報の利用性は格段に進歩しました。遺伝子同定の道筋は明らかになりました。すなわち、これからはどんなサンプルを収集・保有しているかが成否を分けると共に、いよいよ研究者個々人の「研究力」が問われることになります。それらの集合体としての「研究所力」を一段と優れたレベルに維持し、さらに発展させていきたいと思います。

平成18年度の特筆すべき成果は、肉質・肉量に関わる責任遺伝子が絞り込まれただけでなく、黒毛和種牛の繁殖性に関わる遺伝子について有用な情報が得られたことです。乳牛においても２つ目の乳房炎抵抗性遺伝子を同定しました。関係各位におかれましても、研究所の今後の研究推進に一層のご支援をいただければ幸いに存じます。

最後に、終始ご指導いただいた農林水産省、ご理解とご支援を頂いた日本中央競馬会、㈶全国競馬・畜産振興会、および、共同研究を進めてきた機関の各位に厚くお礼申し上げます。

平成19年３月

社団法人畜産技術協会

平成18年度
動物遺伝研究所年報

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第１節　設立の経緯と沿革

1．設立の経緯と沿革

家畜育種の基本は、個体の能力を正確に測定し、遺伝的能力に基づいた選抜を行い、選抜された個体間の交配から次世代を生産するという、一連の作業を反復することにより、望ましい遺伝子型を個体内に集積することにある。

家畜の経済形質の大部分はいわゆる量的形質で、一つ一つは決定的な効果を持たない多数の遺伝子によって支配され、また、遺伝以外の環境などの多くの要因に支配されて形質は発現する。しかしながら、個々の遺伝子を解析することは難しく、そのため、血統情報と表現型に基づいて統計遺伝学的手法により種畜の遺伝的能力を推定し選抜が行われてきた。

統計遺伝学的手法は、1940年代には理論的にほぼ集大成され、近年のコンピュータの発達ともあいまって、BLUPに代表されるような理論と計算手法の発展があり、近年家畜の能力は大きく向上した。とくに乳牛では、年々の遺伝的改良量は加速的に大きくなっている。

しかし、遺伝率が低く、あるいは表現型の測定に多大の時間と経費を要する形質、たとえば、繁殖性、抗病性等の形質については現行の育種法では改良が難しいことが指摘されている。さらに、多様化する育種目標に迅速、的確に対応するためには、育種に要する時間、費用等について効率化が強く求められている。

最近の分子遺伝学並びにその重要な領域であるゲノム研究の進展に伴い、家畜においても遺伝地図の作成が急速に進み、DNAマーカーと経済形質に関与する遺伝領域あるいは遺伝子座（QTL）との連鎖解析が可能になった。連鎖解析が進めば、DNAマーカーを指標として、育種目標に適合した遺伝子型を選抜する新しい育種法の開発が期待できる。また、DNAマーカーを指標とした遺伝性疾病原因遺伝子のキャリアのスクリーニングも可能になる。

我が国の畜産は、外国のそれに比して国土資源の制約、高水準の人件費などきわめて厳しい条件下で低コスト化・高品質化をはからなければならない状況にある。そのためには畜産技術の基本である優良家畜への育種を効率的に行うことが必須であり、上述の新しい育種技術の開発に早急に着手する必要があった。

このような状況から、農林水産省の指導のもとに、日本中央競馬会及び㈶全国競馬・畜産振興会のご理解を得て、日本中央競馬会の畜産振興資金の助成により、㈳畜産技術協会附属動物遺伝研究所が設立されることとなった。

研究の拠点となる建物は平成３年度に設計を開始し、平成４年９月着工、平成５年１月に竣工した。建物は鉄筋コンクリート造り一部２階建て延べ面積884㎡（研究員室、実験室（2)、バイオハザード室、クリーンルーム、ドラフトチャンバー、会議室等）である。さらに、平成6年度にRI実験室、動物飼育室の2室（計116㎡）を増築した。研究プロジェクトの拡大に伴い、実験室が手狭になったことから、平成９年度に新たな実験棟を建設することとなった。平成９年９月着工、平成10年２月竣工で、建物は鉄筋コンクリート造り一部２階建て延べ面積1,094㎡

(DNA解析室、コンピュータ室、大会議室、研究員室、図書保管室等）である。新たな実験棟の建設に伴い、これまでの実験棟を本館、新設棟を別館と呼称している。

研究プロジェクトは、平成4年度から「個体識別システムの開発」、平成６年度から「肉質等経済形質DNAマーカー育種手法開発事業」、平成９年度から「家畜疾病DNAマーカー育種手法開発事業」、平成10年度から「家畜遺伝子解析基盤技術緊急開発事業」及び「食肉品種鑑別技術の確立」が開始されるなど順次拡大されてきた。このうち「個体識別システムの開発」は所期の目的を達成し、平成10年度をもって終了した。このプロジェクトによって数多くのDNAマーカーの開発、遺伝地図上への位置付けを行い、これらのマーカーを適宜選択することにより、個体識別や親子鑑定が実用上支障なくできることを明らかにした。個体識別の手法は、牛肉のトレーサビリティを保証する基本的な技術ともなっている。

これまでに脂肪交雑や枝肉重量に関与するとみられる60個以上の遺伝子座を位置づけ（$p<0.01$)、所在するQTLの遺伝子そのものを同定すべく努力を続けている。遺伝子座が位置づけされたことに基づいて、DNAマーカーを指標にした種畜のスクリーニングが一部で試行されつつある。さらに、黒毛和種、褐毛和種、及び、ホルスタイン種に見られた計5種の遺伝的疾病の遺伝子を特定してキャリアの診断法を開発した。

上記のプロジェクトは平成13年度から新たに、ウシのゲノム地図などの基盤技術の開発や遺伝性疾患のキャリア診断技術の開発などを「畜産新技術開発活用促進事業」として進め、経済形質QTLの特定とこれを活用した育種手法の開発を目標として研究を「肉用牛遺伝資源活用体制整備事業」として進めていたが、平成17年度で終了した。また平成12年度から引き続き「畜産新技術実用化対策事業」の一環としてDNA育種基盤整備事業を進め、この中で従来通り関係する道県との共同研究を推進している。平成14年度からスタートした「BSE生体診断技術緊急開発事業」は平成16年度で終了した。また、平成15年度から「牛肉の品種鑑定技術開発事業」がスタートし、平成17年度で終了した。この事業では、我が国の肉用牛として飼養されている黒毛和種、ホルスタイン種、黒毛和種とホルスタイン種間の交雑種の３者間をDNA情報で鑑別する技術を確立することに成功した。これらの事業はいずれも平成17年度末を終了した。

平成18年度からは新たにウシDNA育種に関する「牛ゲノム有効活用促進事業（肉用牛DNA育種実証事業)」および「牛ゲノム有効活用促進事業（牛有用ゲノム等探索・知的財産化事業)」が始まった。ウシゲノム研究の更なる進展とフィールドでの効果検証に加え、重要な遺伝子情報の特許化を進めることで我が国の畜産業を側面から支援するという責任が課せられた。平成18年度の成果については以下詳述しているが、飼養管理技術の重要性が裏付けられた結果も得られ、興味深い。

職員は平成４年度管理部門２名、研究員２名の計４名から発足し、研究の進展・拡大とともに順次増員し、平成17年度末には所長を含む管理部門３名、研究部門14名（研究員７名、研究補助員７名）となった。

第２節　平成18年度の動き

１．研究推進の状況

１）ウシゲノム解析用ツールの開発

⑴　国内および海外の状況

国際コンソーシアムによるウシ全ゲノム配列の解読の現状について（米国・サンディエゴ、2007年1月）

ウシゲノムの塩基配列決定は、テキサスにあるBaylor医科大のヒトゲノムシーケンシングセンターで行われてきた。まず、2004年９月に、3x（ゲノムの３倍長に相当する長さ）のWGS（Whole Genome Shotgun：全ゲノムを対象にランダムに配列を決定したもの）配列が公開され、ついで、2005年３月に、6x WGS配列が公開された。そして、ついに2006年8月にゲノムドラフト配列が公開された。これは、WGSにBAC skim（フィンガープリントで整列化したBACクローンを、単一、もしくは、プールして読んだ配列）を組み合わせたもので、ゲノムの7.1 倍長の配列（WGS + BAC skim）から作成されている。これまでは、scaffold配列（配列の末端同士の重なりをつないだ配列）という断片の配列が公開されていたが、今回は、29本の常染色体とX性染色体の計30本の染色体の配列として公開された。scaffold配列をウシ染色体上に位置づけるために、統合地図（Composite map）が使用された。これは、当研究所が発表したShirakawa-USDA連鎖地図（4881マーカー）、SUN-RH地図（5513座）、および、SNP連鎖地図（2564マーカー）、BovGen RH地図（9190座）、Illinoi RH地図（3484座）を統合したものである。

ゲノムドラフト配列の完成により、今後は、配列への注釈付け（アノテーション）の作業が本格化すると思われる。また、ドラフト配列の中には、リピート配列などによるつなぎ間違いが残されているので、さらなる正確性の向上も期待したい。

SNPについては、WGS（ヘレフォード種、雄１頭）と、その他６品種（アンガス、ブラーマン、ホルスタイン、ジャージー、リムジン、ノルウエーレッド）のゲノムシーケンシングなどにより、２万個以上のSNPが開発され、これらを用いて各品種のハプロタイプブロック等が解析されつつある。それによると、ウシゲノムの相関解析には30kb間隔のSNP（ゲノム全体で14万個のSNP）が必要で、３染色体（BTA 6, 14, 25）については、この条件を満たすSNPが開発済みとのことだった。現在、Affymetrix社から１万個のSNPを含むDNAチップが市販されているが、Illumina社では、５万個のSNPをタイピングできるDNAチップを市販する予定である。

ゲノムワイド相関解析に寄せる期待は大きく、Kirkpatrickら（Wisconsin大）は、現在市販されている10 K SNPアレイ（Affymetrix社）を用いて、乳牛の双子産率を９家系の父方半きょうだい家系で連鎖-連鎖不平衡マッピングを行い、以前に半きょうだい家系でマッピングした５番染色体の同じ領域にQTLを見出した。また、Georgesら（Liege大）は、Belgian Blue種に見られる劣性遺伝性疾患（Congenital muscular dystonia）のマッピングを300MS（Type I疾患）または3000 SNP（Type II疾患）を用いて行い、各々原因遺伝子のマッピングと変異の同定に成功した。さらに、USDAからは、今後、50Kチップ（Illumina社）を用いて、乳牛種雄牛とアンガス種の選抜集団700頭についてハプロタイプ解析を行い、ゲノム情報による育種選抜を行う計画であるとの報告があった。

⑵ 今後の進め方

これまでに進めてきた高密度ゲノム連鎖地図、および、RH地図とウシ-ヒトゲノム比較地図の

作成はほぼ完成し、現在、国際コンソーシアムでは全ゲノム配列解読を進めている。今後は、興味ある特定の領域からさらに高密度にマイクロサテライトやSNPマーカーを開発することで目的の遺伝子まで到達できるようにしていかねばならない。公表されたウシゲノム配列中に多数のマイクロサテライトが見出される。ゲノム全体から開発されたSNPやヒト・マウスゲノム情報と合わせたウシ遺伝子の情報も蓄積しつつあり、日々更新されている。これらの情報を活用することで、ウシのゲノム解析を進めていく。

2）ウシ遺伝性疾病のDNAマーカー育種手法の開発

⑴ 研究年次：平成９年～平成18年

⑵ 研究目的と期待される成果

ウシの遺伝性疾病の多くは常染色体性単純劣性遺伝病である。これまでに５種の疾病の原因遺伝子同定に成功しており、そのノウハウを生かして引き続き遺伝性疾病のキャリア（当該遺伝子をヘテロに保有する個体）をDNA診断する手法を開発し、発症を防止する。

本事業では、これらの疾病、あるいは、疾病に対する感受性（抵抗性）について、DNAを指標としたスクリーニング手法を開発すると同時に、さらに進んで遺伝性疾病原因遺伝子の単離・特定を行う。このような目的が達成されれば、遺伝子の変異を検出するDNA診断によってキャリアのスクリーニングができるため、遺伝性疾病の発症を制御しつつキャリア牛の遺伝的能力を育種に生かすことができる。

⑶ 研究開発の個別目標と成果

⑶-1 ウシ遺伝性疾病解析の平成17年度までの経緯

本課題は平成９年度から実施しており、当研究所が疾病の原因遺伝子を同定し、DNA診断手法を開発し、家畜改良事業団が検査業務を行うことになっている。平成17年度までの成果を表３に示した。

表３．遺伝性疾病の遺伝子解析のまとめ

劣性遺伝病名	品種	原因遺伝子	変異の種類	遺伝子診断手法	特許
クローディン-16 欠損症	黒毛和種	Claudin-16 （新規）	37kbの欠損	あり	受　理
モリブデン補酵素 欠損症	黒毛和種	MCSU （新規）	３塩基欠損	あり	受　理
Chediak-Higashi 症候群	黒毛和種	CHS-1	１塩基置換	あり	受　理
クローディン-16 欠損症タイプ２	黒毛和種	Claudin-16	56kbの欠損	あり	受　理
軟骨異形成性矮 小体躯症	褐毛和種	LIMBIN （新規）	１塩基置換 １塩基の２ 塩基置換	あり	受　理
横隔膜筋症	ホルスタ イン種	HSP70	11kbの欠損	あり	受　理

黒毛和種では水頭腫、盲目等の遺伝性疾病に取り組んできた。さらに広く疾病の家系を収集するため、道県との共同研究を進めると共に、大学の家畜病院や共済組合などとの連携強化を進めてきた。

⑶-2 ウシ遺伝性疾患の解析に関わる平成18年度の成果

⑶-2-1. 乳頭欠損症（県との共同研究）

先天的乳頭欠損症（ATPP: Abnormal Teat Patterning Phenotype）が、特定の黒毛和種種雄牛の産子の性別に関わらず多発した。乳頭１対の２本欠損、１本欠損、乳頭形状の異常など多様な表現型を示した。乳頭欠損を伴う雌個体は繁殖雌牛として登録できないため、農家は大きな経済損失を被っている。そこで、発症をコントロールする診断手法の開発を目的として原因遺伝子座のマッピングを行った。その結果、17番染色体セントロメア側（*ATPP1*:p=2.2x10^{-9}）および１番染色体のセントロメア側（*ATPP3*:p=0.035）とテロメア側（*ATPP2*:p=0.005）の計３カ所に父由来アリル頻度が有意に異なる領域が見いだされた。２本欠損個体のリスク比は、*ATPP1*=2.2、*ATPP2*=1.7、*ATPP3*=1.6で*ATPP1*が発症に対し最も大きく寄与していた。*ATPP1*は１本欠損個体（48頭）においても有意であった（リスク比: 1.9、p=2.3x10^{-4}）。これら３遺伝子座すべてにおいてリスクハプロタイプを排除すれば、乳頭欠損個体の発症確率を0.06にまで下げることができる。

ゲノム解析で領域を狭めることは困難なので、ヒト-マウス-ウシ間のゲノム比較地図を詳細に調べ、ウシの３領域に該当するマウス領域を決定した。マウス新生児の乳頭部位において皮膚組織と比べ特異的な発現をしている遺伝子を、マウスcDNAマイクロアレイを用いて調べた。その内、該当するマウス領域に存在する遺伝子群に絞った。今後、ウシ新生児からの組織を使い、発現の違い、塩基配列の違いなどを指標に原因遺伝子を捜す。さらに、農家が保留を希望する雌牛について、リスクハプロタイプの有無を調べる予定である。

⑶-2-2. 眼球形成不全症（岡山大学との共同研究）

眼球形成不全症（Multiple Ocular Defects, MOD）を発症した18頭を中心とする集団の連鎖解析を行い、18番染色体の領域にマッピングした。常染色体劣性遺伝病であった。その後、岡山大では解析を進め、原因遺伝子の同定に成功し、DNA診断法を確立した（未発表）。

⑶-2-3. 前肢帯筋異常症（岡山大学との共同研究）

黒毛和種に見られる前肢帯筋異常症（Forelimb-girdle Muscular Anomaly）の解析を行った。同一地域の集団においてこれまでに２回発症牛が頻発したが、交配の調節で対処してきた。しかしながら、最近、頻発が繰り返された結果、ゲノム解析での解決を図ることとした。父方半きょうだい家系（３頭の種雄牛を含む）に属する26頭の発症牛を集め、DNAを調製した。全染色体上に配置した258のマイクロサテライトマーカーを用いて発症との関連を調べたところ、26番染色体のテロメア側にあるマーカー群が発症と有意に連鎖していることがわかった。そこで、この領域にさらにマーカーを追加しハプロタイプを検討した結果、候補領域を10cMにまで絞り込むことができた。ヒトあるいはマウスとの比較地図から、この領域にはN-RAPやPDZ8など原因遺伝子となりうる複数の候補遺伝子が含まれている。

⑷ 国内および海外の状況

Georgesら（Liege大）は、Belgian Blue種に見られる劣性遺伝性疾患（Congenital muscular dystonia）のマッピングを300 MS（Type I疾患）または3000 SNP（Type II疾患）を用いて行い、各々原因遺伝子のマッピングと変異の同定に成功した（未発表）。

⑸ 今後の進め方

眼球形成不全症は過去に他の地域で頻発していた遺伝病であり、また、前肢帯筋異常症も過去に頻発していたものである。ゲノム解析の手法を使うことで問題は解決されることを今後も示していく。

3）肉用牛経済形質のDNA育種手法の開発

⑴ 研究年次：平成６年～平成18年

⑵ 研究目的と期待される成果

ウシの経済形質の改良はこれまで主としてBLUP等に代表される統計遺伝学的手法によって行われており、大きな成果を挙げてきた。しかしこの方法では種畜評価に要する時間、コストが膨大なものになる欠点がある。一方、近年におけるゲノム解析研究の進展は、ゲノム連鎖地図を用いることにより、特定経済形質に関与する染色体上の遺伝領域、あるいは遺伝子を特定することを可能にしつつある。しかしながら、ウシのほとんどの経済形質は量的形質であるため、責任遺伝子の特定は困難であることが容易に予想できる。責任遺伝子の特定という目的を達成するには明確な戦略に基づいた組織的・継続的な取り組みが欠かせない。経済形質を支配する領域や遺伝子座を同定することでDNA情報を活用した育種が可能になるだけでなく、飼養管理技術の改善に結びつくことが望ましい。

⑶ 研究開発の個別目標と成果

⑶-1 経済形質解析のためのDNAサンプルの収集

平成13年度から20道県（21道県、平成18年度）・家畜改良事業団・家畜改良センターと共同研究を実施している。特定種雄牛を父とする大規模な父方半きょうだい家系を作成することは、道県においては該当種雄牛の遺伝的能力の的確な把握と後継種雄牛の作成に重要であり、かつ、多種多様な解析用家系の作成は経済形質に影響する遺伝子（QTL）を同定するためにも有用である。そこで、枝肉共励会や枝肉共進会等において血統情報の明らかな肥育牛のDNAサンプルを収集することを始めた。当研究所では、東京食肉市場、および、大阪市食肉市場におけるサンプリングを実施している。平成18年度までの収集の状況は表１の通りである。これらの収集した黒毛和種のDNAサンプル数は約２万４千となった。

表１．平成18年度までの黒毛和種DNAサンプル収集状況

年　　度	収　集　数
13	1,054
14	2,503
15	5,387
16	4,922
17	4,634
13	5,827
合　　計	24,261

⑶-2 ウシ経済形質解析のまとめ

経済形質（QTL）の解析において、染色体毎（染色体ワイズ、chromosome-wise）やゲノム毎（ゲノムワイズ、experiment-wiseまたはgenome-wise）に有意水準を検定し、多重検定を補正するインターバルマッピング法であるQTL Express（Haleyら、1994；2002）が優れている。肉牛の経済形質のような量的形質には、複数の遺伝子座の間の相互作用であるエピスタティック（非相加的）効果が見られる場合がある。そこで、相互作用も検証するため、我々はQTL Express を改良したGlissardoを開発した。平成18年度までに全国で行った家系解析の結果を当研究所にて確認作業を行い、15家系についての経済形質マッピングについてまとめ、論文発表した（研究発表の章参照：Takasuga,A., Watanabe,T., Mizoguchi,Y., Hirano,T., Ihara,N., Takano,A., Yokouchi,K., Fujikawa,A., Chiba,K., Kobayashi,N., Tatsuda,K., Oe,T., Furukawa-Kuroiwa,M., Nishimura-Abe,A., Fujita,T., Inoue,K., Mizoshita,K., Ogino,A., Sugimoto,Y. (2007) Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping. *Mammalian Genome,* 18: 125-136.）。それらの結果を表２に示す。

表２．平成18年度までの黒毛和種経済形質マッピングのまとめ

経済形質QTL*	$p < 0.01$	$p < 0.001$
体重	9	2
枝肉重量	13	2
脂肪交雑	19	9
ロース芯面積	13	3
バラ厚	11	1
皮下脂肪厚	18	3
合計	83	20

*染色体ワイズの有意水準。

⑶-3 ウシ経済形質遺伝子座のポジショナルクローニングに関わる今年度の成果

⑶-3-1. *Marbling-1*（脂肪交雑-1）の解析（兵庫県との共同研究）

これまでに、父方半きょうだい家系を用いた連鎖解析を行い、21番染色体テロメア側に脂肪交雑連鎖領域3.5cMを特定し、２種類の集団で母方アリルについての相関解析により約720kbに限局化した。この領域に存在する12種の遺伝子にアミノ酸変異は認められなかったが、３種の遺伝子は筋間脂肪においてq/-と比べQ/qの発現量が22-94倍高かった。*Q/q*最長筋ロースのバイオプシーを月齢18ヶ月から30ヶ月まで２ヶ月毎に行い、経時的発現量の経緯を調べたところ、脂肪部位で28ヶ月と30ヶ月の間で急激な増加を示した遺伝子aが有力な候補となった。

平成18年度は、ウシ脂肪細胞前駆細胞株（東北大学麻生先生により樹立）の脂肪細胞分化系を用いた解析を行った。遺伝子aについて有力な結果が得られたので、遺伝子a領域のすべての一塩基多型（SNP）を探索し、アリル頻度から責任SNPを特定する。

⑶-3-2. *Marbling-2*（脂肪交雑-2）の解析（宮崎県・岐阜県との共同研究）

平成17年度までに、SNPの検出で約50kbの領域まで狭めたが、可能性のあるセントロメア側の４cMを除外する根拠があまりないこと、優良遺伝子型Qの頻度が低いため、領域を狭めることは難しいことがわかった。

平成18年度は、4cMの領域にDNA tiling array（ゲノム上に相補的な25塩基のプローブを平均150塩基のギャップを持たせて並べたもの、RNAに転写された領域を実験的にサーチできる）を作成し、ロース部位における遺伝子発現の*Q*vs.*q*比較を行った。この手法は網羅的な発現解析に優れていると言われており、データの解析を急いでいる。

⑶-3-3. *CW-1*（枝肉重量-1）の解析（鹿児島県との共同研究）

平成17年度までに、黒毛和種父方半兄弟家系を用いた連鎖解析により、枝肉重量QTL（*CW-1*）を14番染色体セントロメア付近8.1cMの領域にマップし、相関解析で1.1Mbに狭めた。本領域内に含まれるすべての遺伝子（４種）についてそれぞれBACクローンを単離し、BACクローン導入マウス（トランスジェニックマウス: Tg マウス）の作成を開始した。

平成18年度は、Tg マウスの作成をしつつ、CW-1領域を解析した結果、（i） この領域は729kb、（ii） 候補遺伝子の１つは領域外、（iii） 遺伝子の存在しない２つの領域（114 kb 領域、308kb領域）の存在がわかった。この729kbについてDNA tiling arrayを作成し、RNA転写の有無と発現の*Q*vs.*q*比較を行った。データを解析している。今年度後半になってTg マウスの作成がほぼ終了したので、Tg マウス毎に成長実験を開始した。雄・雌それぞれ個体数６匹以上の集団を20週まで調べる予定である。

⑷ 国内および海外の状況

肉牛については、米国などから経済形質のマッピングについてこれまでにいくつか報告されているが、ゲノムワイズレベル１％以下、20cM以内にファインマッピングされている例はまだない。平成18年度においても顕著な報告は見られなかった。

⑸ 今後の進め方

Marbling-1、*Marbling-2, CW-1*遺伝子を同定するため、候補遺伝子の機能の解明を培養細胞レベル・マウス個体レベルで進め、遺伝子機能の解明から責任遺伝子の同定を行う。

４）ウシ抗病性遺伝子座の解析

⑴ 研究年次：平成７年～平成18年

⑵ 研究目的と期待される成果

小型ピロ、脂肪壊死症、乳房炎等の疾病に対する感受性（抵抗性）は、単純劣性遺伝ではないものの、遺伝的変異のあることは明らかであり、これらの疾病に対する感受性（抵抗性）についてDNA診断でスクリーニングできれば経済的に重要な意義を持つこととなる。これらの内、平成18年度には乳房炎について更なる進展があった。

⑶ ウシ抗病性遺伝子座の解析に関わる今年度の成果

⑶-1. 乳房炎抵抗性の解析（家畜改良センター・北海道との共同研究）

牛群検定に参加した搾乳牛の初産時体細胞数を乳房炎抵抗性の指標とした。特定地域で飼養され、共通の祖父牛由来の集団から、体細胞数が低い集団（< 25,000; 297頭）と高い集団（> 100,000; 181頭）を収集し、連鎖解析した結果、染色体21番と22番に有意な連鎖を見いだした。

平成17年度までに、染色体22番のQTL（SCC_{22}）について、グリシン残基１個の挿入変異の認められた*FEZL*（*Forebrain embryonic zinc finger-like*）を責任遺伝子とした。挿入変異のある*FEZL*遺伝子は感受型（*S*）で、そうでない方は抵抗型（*R*）であった（特許申請中）。また、染色体21番のQTL（SCC_{21}）について、候補遺伝子のプロモーター活性を支配するSNPを見いだした。

平成18年度は、培養細胞系を用いた実験から、SCC_{21} 候補遺伝子のFEZLによる発現調節を明らかにした。プロモーター部位のSNPは抵抗性と連鎖しており、培養細胞系を用いた実験で細菌の捕食性に影響することがわかった。第２の乳房炎抵抗性遺伝子についても特許申請中である。平成18年度後半から、家畜改良センターの牛群を使った効果検証とDNA情報の実用化のためのプロジェクトを開始した。

⑷ 国内および海外の状況

これまで、米国農務省、イリノイ大学、ノルウェー農業大学などから、体細胞数を指標とした乳房炎抵抗性のマッピングが報告されてきたが、ファインマッピングの段階には至っていない。

⑸ 今後の進め方

日本ホルスタイン登録協会などと共同研究を行い、ホルスタイン種種雄牛の遺伝子型と搾乳牛の乳房炎発症率についての相関性を調べる。また、抵抗性遺伝子型ホモの種雄牛を作成し、効果検証に用いる。

黒毛和種の脂肪壊死に関しては、ウシ全ゲノムを対象とする相関解析を実施すべき状況を調えているところである。平成19年度には解析を開始する。

５）ウシ全ゲノムを対象とする相関解析

⑴ 研究年次：平成18年～平成20年

⑵ 研究目的と期待される成果

これまでウシ経済形質のゲノム解析では、父方半きょうだい家系を作成し、マイクロサテライトのような多型性の高いDNAマーカーの型判定結果に基づいたQTL解析を行ってきたが、国際コンソーシアムによるウシゲノム解読の進展により、新しい解析手法が確立しつつある。すなわち、多数の一塩基多型（SNP）を用いたゲノムワイド相関解析である。牛有用性ゲノム等探索・知的財産化事業の開始に伴い、新手法を活用しつつ、これまで解析の困難であった表現型も解析の対象に加え、有用なDNA情報を探索していく。

⑶ ウシ全ゲノムを対象とする相関解析の試み～過排卵処理への反応性（全農ETセンター、家畜改良センターとの共同研究）

黒毛和種牛では、遺伝的な能力の高い雌牛をFSHなどで過排卵処理して採卵し、受精卵として販売されている。しかしながら、購入した雌牛から採卵できない場合がある。あらかじめ採卵

性の高い牛をDNA情報で選抜する手法の確立が期待されている。昨年度までに、家系の構築に拠らない全ゲノムを対象とする相関解析手法を過排卵処理への反応性に適用してきたが、いくつかの問題点がわかり、平成18年度に全面的なやり直しを行った結果、有力な候補遺伝子に到達することができた。

５回以上の採卵データのある約500頭の黒毛和種メスから採卵数の上位および下位、各42頭ずつを抽出し、常染色体１～29番およびX染色体に配置した約1,000個のマイクロサテライトマーカーの型判定を行った。その結果、22個の染色体で5%水準（多重検定補正済み）で有意な領域を得た。二次スクリーニングで上位下位67頭ずつ使い、９個の染色体で有意な結果を得た。最も相関していた領域を多数のマイクロサテライトで解析したところ、約160kbの領域が有意となった。この領域には遺伝子が１個存在し、塩基配列を調べたところ、アミノ酸変異が検出された。この変異と繁殖性との関連を今後調べていく予定である。

⑷　国内および海外の状況

連鎖不平衡は、これまで、ベルギーやイスラエルで経済形質のファインマッピングの手法として使われてきた。Kirkpatrickら（Wisconsin大）は、現在市販されている10K SNPアレイ（Affymetrix社）を用いて、乳牛の双子産率を9家系の父方半きょうだい家系で連鎖-連鎖不平衡マッピングを行い、以前に半きょうだい家系でマッピングした５番染色体の同じ領域にQTLを見出した。全ゲノムを対象とする相関解析は期待される手法である。

⑸　今後の進め方

過排卵処理への反応性については、責任遺伝子の同定を視野に入れて進める。黒毛和種における脂肪壊死症抵抗性についてのサンプリングは進んでいる。また、ホルスタイン種における受胎率についてのサンプリングを平成19年度１月から始めた。５年間くらい継続する。黒毛和種の受胎率では、サンプリングを開始した。サンプリングを充実させ、今後のゲノムワイド相関解析につないでいく。

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３）学会発表要旨

[学会発表の次の数字は、学会発表の番号に相当する]

学会発表１.

題　目：Fine mapping and gene discovery in cattle.（ウシにおけるファインマッピングと遺伝子同定）

発表者：渡邊　敏夫、杉本　喜憲

所　属：畜技協・動物遺伝研

要　旨：家畜ゲノム研究の主な目的は生産形質のバリエーションに対して遺伝的な要因がどのように寄与するかを理解することにある。ウシにおいては経済的に重要な形質の多くは量的形質遺伝子座（QTL）により支配される。われわれは15の純粋黒毛和種の父方半きょうだい家系を構築し、染色体ワイズ１％有意なQTLを60以上検出した。これらのQTLのうちいくつかは複数の家系において同一染色体領域にくり返し検出された。（染色体14番における枝肉重量QTL、染色体４番、21番における脂肪交雑QTLなど。）われわれは連鎖不平衡マッピングとIBD解析の手法を用いてファインマッピングをおこなった。染色体14番における枝肉重量のファインマッピングは成功しており、染色体21番におけるそれもよい結果を得ている。われわれが置かれているウシQTL解析の現状をのべ、その研究の進捗について報告をする。

（第8回世界家畜生産応用遺伝学会議、2006年８月、ブラジル）

学会発表２.

題　目：Detection of epistatic QTLs for beef marbling by bayesian MQEM method in Japanese Black cattle.（ベイズMQEM法による黒毛和種における脂肪交雑エピスタシスQTLの検出）

発表者：T.Miyake[1], A.Narita[1], M.Kurosawa[1], H.Nakaoka[1], T.Watanabe[2], K.Yokouchi[2], Y.Sugimoto[2], M.Ito[3], T.Fujita[3], T.Yamada[1], Y.Sasaki[1].

所　属：[1]京大院農、[2]畜技協・動物遺伝研、[3]大分農林水産研究セ

要　旨：経済形質の多くは量的形質遺伝子座（QTL）により支配されるが、これらのQTL間の相

互作用（エピスタシス）が表現型に重要である可能性がある。黒毛和種半きょうだい家系における脂肪交雑形質に対するQTLの主効果とエピスタシス効果をMCMCによるベイズ推定（MQEM）により検出することを本研究の目的とした。MQEMは古典的なインターバルマッピングよりも多くのQTLを検出することに成功し、さらに一つのエピスタシスQTLペアを検出した。この結果はMQEM法がQTLの主効果およびエピスタシス効果を同時に検出するのに有効であることを示している。
（第８回世界家畜生産応用遺伝学会議、2006年８月、ブラジル）

学会発表３.
題　目：Whole-genome LD mapping of bovine QTL that control superovulatory response of ovary using microsatellite markers.（マイクロサテライトマーカーを用いたウシ過排卵処理反応性QTLの全ゲノムLDマッピング）
発表者：T.Watanabe[1], A.Ideta[2], Y.Mizoguchi[1], Y.Aoyagi[2], Y.Sugimoto[1]
所　属：[1]畜技協・動物遺伝研、[2]全農ETセ
要　旨：過排卵処理反応性に関する遺伝因子を探索し、ウシの繁殖製を向上させる目的で、マイクロサテライトマーカーを用いた全ゲノムを対象としたQTLのLDマッピングをおこなった。FSHにより過排卵処理をおこなった703頭の黒毛和種メスから表現型値が両極端のもの、すなわち排卵数の平均値が上位のものと下位のもの96頭ずつを選択した。染色体１～29番およびXを対象に1073個のマイクロサテライトによりこれら個体の型判定をおこなった。上位および下位集団における隣接する２マーカーからなるハプロタイプの頻度推定をそれぞれおこない、Fisher正確検定によりハプロタイプの偏りを検定した。17カ所において有意な結果（$P < 10^{-3}$）を得、うち７ヵ所おいてはP ＜ 4.8 x 10-5となる非常に有意な結果を得た。６ヵ所についてマーカーを追加して検査したところ、うち４ヵ所について強い相関が維持された。興味深いことにこのうち２ヵ所はプロスタグランジン合成経路に関わる遺伝子座の近傍であった。プロスタグランジンは卵成熟、排卵、マウスにおけるリッターサイズに関与することがすでに報告されている。われわれは家畜動物種においてはじめてマイクロサテライトによるQTLの全ゲノムLDマッピングに成功した。これらのマーカーは過排卵処理反応性に関するより正確な選別基準を与えうるであろう。
（国際動物遺伝学会第30回大会、2006年８月、ブラジル）

学会発表４.
題　目：黒毛和種の枝肉重量および枝肉形質のQTL地図
発表者：高須賀晶子[1]、渡邊敏夫[1]、溝口　康[1]、平野　貴[1]、井原尚也[1]、高野　敦[1]、横内　耕[1]、藤川　朗[2]、千葉和義[3]、小林直彦[4]、龍田　健[5]、小江敏明[6]、古川　恵[7]、安部亜津子[8]、藤田達男[9]、井上和也[10]、溝下和則[11]、荻野　敦[12]、杉本喜憲[1]
所　属：[1]畜技協・動物遺伝研、[2]北海道畜試、[3]宮城県畜試、[4]岐阜県畜産研、[5]兵庫県農水総技セ、[6]鳥取県畜試、[7]岡山県総畜セ、[8]島根県畜技セ、[9]大分県農水研セ畜試、[10]宮崎県畜試、[11]鹿児島県肉改研、[12]家畜改良事業団
要　旨：【目的】多くの父方半きょうだい家系を解析することによって、黒毛和種の枝肉重量や脂肪交雑などの経済形質に重要なゲノム領域を明らかにし、責任遺伝子の同定や種雄牛の育種選抜に利用できるマーカー情報を蓄積する。

【材料と方法】動物遺伝研と各道県試験場および家畜改良事業団との共同研究により、15の大規模な父方半きょうだい家系（産子数190頭以上）をゲノムワイドでQTL解析を行った。QTL解析には、線形回帰によるインターバルマッピングの手法を用い、有意水準は、染色体毎にpermutation testにより算出した。複数形質についての多重検定による偽陽性の混入を防ぐ目的で、False discovery rate（FDR）を計算した。95%信頼区間の計算は、Bootstrap法によった。検出したQTLは、適宜、マーカー数と産子数を増やして２次スクリーニングを行った。また、同じ形質のQTLが複数家系で同じ領域にマップされているものについて、共通の優良型(*Q*)ハプロタイプを持つか否かを検討した。

【結果】15家系の産子数は、192頭から872頭（２次スクリーニングを含む)、総数7860頭であった。検出されたQTLのうち、「有意な連鎖」（ゲノムレベル５％有意、または２次スクリーニングで染色体レベル0.01％有意を超えたもの。FDRは0.1以下）は40個あり、複数家系で重複して検出されたものも多く含まれていた。また、各QTLの寄与率は2-13%であった。

14番染色体（BTA 14）の枝肉重量QTLは、５家系で「有意な連鎖」を示し、他の２家系で染色体レベル１％有意で検出された。これら７家系の種雄牛は、1.1Mbの*Q*ハプロタイプを共有しており、これは、以前に報告した、連鎖不平衡マッピングで狭めた領域と一致した。

このほか、「有意な連鎖」を示す、BTA ４の脂肪交雑QTLとロース芯面積QTL、BTA 14のロース芯面積QTLも複数家系で検出された。また、BTA 6、7、9、10、20、21の脂肪交雑QTLと、BTA 6の枝肉重量QTLは、他の家系で、染色体レベル１％または５％有意で検出された。これらのうち、BTA ６の脂肪交雑QTLは*Q*ハプロタイプが共通であり、BTA 4と10の脂肪交雑QTLは*q*ハプロタイプが共通であった。

（国際動物遺伝学会第30回大会、2006年8月、ブラジル）

学会発表５.

題　目：４番染色体上脂肪交雑QTL（*Marbling-3*）の相関解析によるファインマッピング

発表者：横内　耕[1]、溝口　康[1]、岩本英治[2]、渡邊敏夫[1]、高須賀晶子[1]、杉本喜憲[1]

所　属：[1]畜技協・動物遺伝研、[2]兵庫県農水総技セ

要　旨：BMS（脂肪交雑等級）は肉用牛の重要な経済形質であるが、その責任遺伝子はまだ報告がない。我々は以前、黒毛和種1家系（N=872）の半兄弟家系解析から、脂肪交雑QTLを４番染色体上の35-85cMに検出した（ISAG2004)。

今回、この種雄牛Aとは兄弟牛である種雄牛Bについて、同様に半兄弟家系（N=341）を構築し、４番染色体上の37のマイクロサテライト（MS）マーカーを用いて家系解析を行った。その結果、やはり有意な脂肪交雑QTLが30-80cMに検出され、４番染色体中央部に共通の脂肪交雑QTLが位置していると予想された。

そこで、40から80cMの領域に新規にMSマーカーを開発し、計83のMSマーカーを用いて種雄牛Aと種雄牛Bのハプロタイプを比較した。種雄牛Bは種雄牛Aと共通の*q*を父牛から受け継いでおり、優良*Q*はいずれも母牛由来であることがわかった。さらに、これらの母牛は同じ県内の黒毛和種繁殖雌牛集団に属するにも関わらず、両*Q*の間で５cM以上の広範囲に渡って共通している領域は認められなかった。この脂肪交雑QTLが県内の共通祖先牛由来（IBD）であるとすると、Qの中のより狭い共通領域に含まれていると考えられる。

次に、黒毛和種集団における種雄牛Bの*Q*と脂肪交雑との関連について検討する目的で、相関解析を行った。対象として、両母牛が属する同じ県内市場に出荷された黒毛和種肥育牛307頭

(BMS≧8,116頭;BMS≦4,191頭）を用いた。40から80cMの領域に配置した多型性の高い53のMSマーカーを用いて型判定を行い、各*Q*アリルとBMSとの相関を検定した。その結果、*Q*とBMSが最も強く相関しているMSマーカー（P=0.0058）は、*q*とは異なり、半兄弟家系解析において最大のスコアが検出された領域に位置していた。さらに、このMSマーカーは*Q*が共通な10の連続するMSマーカーの一つであって、それにより構成される２cMの共通領域は集団内でLDブロックを形成していた。現在、われわれはさらなる解析のため、LDブロック内にある既知遺伝子周辺のSNPを検索中である。
（第30回国際動物遺伝育種学会大会、2006年８月、ブラジル）

学会発表６.
題　目：第２の乳房炎抵抗性遺伝子の同定
発表者：杉本真由美[1]、杉本喜憲[2]
所　属：[1]家畜改良セ、[2]畜技協・動物遺伝研
要　旨：Walkway chief markを祖父とする６半兄弟種雄牛家系の娘牛478頭について初産次平均体細胞数との連鎖解析を行った結果、第21番染色体にLOD scoreが28という強い連鎖が見られた。第９回及び第10回学術集会において発表したとおり、当該家系ではforebrain embryonic zinc-finger like（FEZL）遺伝子のグリシン鎖において12個のグリシン（12G）が体細胞数の高い個体では13個（13G）となる多型が見いだされ、転写因子であるFEZLはInterleukin-8等のサイトカインの発現を高めることが確認できている。そこで、第21番染色体における乳房炎抵抗性遺伝子がFEZLの影響を受けていないかどうか調べるため、12G/12GFEZL、12G/13GFEZL、13G/13GFEZLを持つ個体の３群に分けて連鎖解析を行ったところ、13G/13GFEZLを持つ個体において、解析頭数が478頭から224頭に減るにもかかわらず、LOD scoreが35というより強い連鎖が見られた。この結果は、第21番染色体における乳房炎抵抗性遺伝子はFEZLによる発現調節を受けていることを示している。Microarray解析、real-time PCR及びluciferase解析により、第21番染色体上に位置する遺伝子のうち、insulin-like growth factor 1 receptor（IGF1R）遺伝子がFEZLによる発現調節を受け、さらにそのpromoter領域において体細胞数の高い個体ではシトシンの数が増えるという多型を見いだした。IGF1RはFEZLとともに、乳房炎抵抗性に関与すると考えられる。
（日本乳房炎研究会第11回学術集会、2006年10月、つくば）

学会発表７.
題　目：熊本系褐毛和種集団の枝肉重量およびロース芯面積に関する多座位相関解析
発表者：渡邊 真司[1]、小邦 朋子[2]、松本 道夫[2]、渡邊 敏夫[3]、横内 耕[3]、杉本 喜憲[3]、三宅 武[1]、山田 宜永[1]、佐々木 義之[4]
所　属：[1]京大院農、[2]熊本農研セ畜研、[3]畜技協・動物遺伝研、[4]ビッグ研究所㈱
要　旨：【目的】近年、育種改良にQTL情報を用いることが注目を集めており、多くの経済形質に関するQTLが染色体上に特定されてきている。演者らは、熊本系褐毛和種集団の枝肉重量およびロース芯面積に関するQTLをBTA6の58cM～106cMの領域に推定した（日本畜産学会第106回大会)。そこで本研究では熊本系褐毛和種集団の連鎖不平衡を利用し、推定されたQTL領域を狭めることを目的とした。
【方法】材料として、１頭の種雄牛から由来する半きょうだい320頭およびBTA 6の51cM～111cM

に散在する18個のマーカー型情報（平均マーカー間隔は3.4cM）を用いた。表現型値として枝肉重量とロース芯面積を用い、320頭の表現型値は肥育牛55,513頭のフィールド記録からBLUP法によりBLUE値を用いて補正した。組換え率を利用して父方由来配偶子を100回反復推定し、マーカー型に基づいて判定される母方由来配偶子を100セット得た。解析では５％以下の頻度であったマーカーアリルを欠測値として処理した。多座位相関解析にはKilpikariとSillanpaa（2003）の方法を用い、100万回のMCMCサイクルのうち100回に１回サンプルを抽出した。100セットの母方由来配偶子について解析した結果を平均し、事後確率を推定した。
【結果】表現型と相関のあるマーカー座数に関する事後確率から枝肉重量では２個のマーカー座を含むモデルが37.1％、およびロース芯面積では１個のマーカー座を含むモデルが46.3％と全体で最も多かった。本研究では、枝肉重量では60.2cMと54.2cMおよびロース芯面積は85.8cMのマーカー座の近傍にQTLが存在すると考えられた。しかし、データセットごとにモデルに取り込まれた候補マーカー座の順位が上下し、正確度についてはさらなる検討が必要だと考えられた。
（第７回日本動物遺伝育種学会大会、2006年11月、鹿児島）

学会発表８.
題　目：大分県産黒毛和種の父方半きょうだい家系を用いたQTL検索
発表者：藤田達男[1]、横内　耕[2]、渡邉直人[1]、佐藤文明[1]、伊藤雅之[1]、伊東克久[1]、渡邊敏夫[2]、杉本喜憲[2]
所　属：[1]大分農林水産研畜産、[2]畜技研・動物遺伝研
要　旨：【目的】大分県産黒毛和種の育種改良に応用可能な効率的で精度の高いDNA育種手法の実用化に向けて、肉用牛経済形質に関与する遺伝子座を検出する。
【方法】本県を代表する種雄牛「糸福」の半きょうだい家系（去勢肥育牛229頭）について、250マーカーの遺伝子型データと産肉６形質（枝肉重量（CW)、日増体量（DG)、ロース芯面積、バラの厚さ、皮下脂肪の厚さ、BMS.ナンバー(BMS)）の枝肉データまたは育種価の２通りの表型値を用いてQTL解析プログラムGlissadoで解析。育種価は、母数効果として市場、出荷年度および肥育農家または地域の効果を、共変量として肥育期間および肥育期間終了時日齢への２次までの回帰の効果を考慮。CWとDGに陽性効果のあったBTA 8、BMSに陽性効果のあったBTA 14、BTA 18およびBTA 21に更にマーカーを追加し、合計275マーカーを用いて２次スクリーニングを実施。
【結果】Genome-wise ５％水準で有意なQTLを、枝肉データを用いた解析で23領域、育種価を用いた解析で21領域検出。この内Lod scoreが10.5以上のQTLは８領域あり、これらは枝肉データと育種価の両方の解析結果に共通して検出。BMSに陽性効果のあったBTA 14のQTLは、２次スクリーニングで消滅。CWとDGに陽性効果のあったBTA 8のQTLは共通領域（80-120cM）にあり、これらのアリル置換効果は枝肉データで30kg、89g、育種価で12.4kg、31g。BMSに陽性効果のあったBTA 18（70cM～テロメア側)、およびBTA 21（40-60cM）の両QTLのアリル置換効果は、それぞれ枝肉データで1.07、1.00、育種価で0.7、0.49。BTA 18とBTA 21の両QTLを組み合わせたBMSのアリル置換効果は相加的で、枝肉データで2.1、育種価で1.14であった。
（第７回日本動物遺伝育種学会大会、2006年11月、鹿児島）

学会発表９.
題　目：鹿児島黒牛における枝肉重量遺伝子座の効果検証

発表者：瀬戸口浩二[1]、高野　淳[2]、溝下和則[3]、山口　浩[1]、渡邊敏夫[2]、杉本喜憲[2]
所　属：[1]鹿児島県・肉改研、[2]畜技協・動物遺伝研、[3]鹿児島県・南薩家保
要　旨：【目的】鹿児島黒牛の経済形質に関する遺伝子座を同定し、種雄牛選抜の指標の一つとして利用するために、父方半兄弟家系を用いたQTL解析を行っている。これまでに我々は、県有種雄牛AおよびBの各々の家系解析において、枝肉重量QTL（*CW-1*、*CW-2*）を14番および６番染色体上にマップした。今回は*CW-1*、*CW-2*ともに優良遺伝子型*Q*をヘテロに持つ県有種雄牛C（sireC）の産子を用いて、これらの遺伝子座の効果検証を行ったので報告する。
【方法】*CW-1*、*CW-2*領域に各々20個のマイクロサテライトマーカーを配置し、sireCの産子（去勢肥育牛）289頭についてマーカーの型判定を行った。*CW-1*、*CW-2*各々において、*Q*ホモ（*Q/Q*）、*Q*ヘテロ（*Q/-*）、non-*Q*（-/-）に分類し、*t*検定を実施した。
【結果】*CW-1*における遺伝子型の分布は*Q/Q*：92頭、*Q/-*：137頭、-/-：59頭と、*Q*タイプが高頻度に保存されていることが分った。このときの平均枝肉重量は*Q/Q*：441.8kg、*Q/-*：425.1kg、-/-：399.0kgとそれぞれに有意差（$P < 0.01$）がみられた。一方で、*CW-2*における遺伝子型の分布は*Q/Q*：29頭、*Q/-*：124頭、-/-：111頭であった。平均枝肉重量は*Q/Q*：445.0kg、*Q/-*：432.1kg、-/-：410.7kgで、*Q/Q*、*Q/-*と-/-間で有意差（$P < 0.01$）がみられた。
　CW-1、*CW-2*両領域が*Q/Q*である産子、non-*Q*である産子集団間における枝肉重量の差は60.6kg（$P < 0.01$）と、相加的な効果を持つことが明らかとなった。従って、これら２つの*Q*ハプロタイプの選抜が、枝肉重量の育種改良に役立つ可能性が示唆された。
（日本動物遺伝育種学会第7回大会、2006年11月、鹿児島）

学会発表10.
題　目：黒毛和種父方半きょうだい家系によるQTL解析
発表者：平野　貴[1]、井上和也[2]、渡邊敏夫[1]、杉本喜憲[1]
所　属：[1]畜技協・動物遺伝研、[2]宮崎県畜試
要　旨：【目的】家畜の経済形質の多くはQTLであり、これらを特定することでマーカーアシスト選抜を利用した効率的な育種が可能となる。我々は黒毛和種の肉質（特に脂肪交雑）に関するQTLを特定し、そのDNA情報を育種選抜に利用するために、黒毛和種肥育牛の大規模な父方半きょうだい家系を用いてQTL解析を進めている。今回は、宮崎県で供用されている種雄牛の父方半きょうだい家系を用いた解析の結果を報告する。
【方法】解析に用いた父方半きょうだい家系は231頭の去勢肥育牛からなり、これらに対して全常染色体をカバーする240個のマイクロサテライトマーカーの型判定を行った。ゲノミックDNAは、枝肉共進会で採材された全血または腎周囲脂肪から抽出した。得られた各枝肉成績の中で、体重、枝肉重量、ロース芯面積、皮下脂肪厚、BMS、バラ厚を表現型としてQTL解析を行った。各Genotypeは、ABI3700シークエンサーで電気泳動した後GenescanおよびGenotyperソフトウェアによって決定した。
【結果】今回の解析により、<５％Chromosome-wise レベルで11カ所のQTLが検出された。特に、BTA20のBMSに関するQTLとBTA14のバラ厚（cm）に関するQTLは、<５％Experiment-wiseと強い有意水準で検出された。それぞれのアレル置換効果は0.98と0.5であり、寄与率は5.6％と9.8％だった。BMSに関するQTL領域について近縁種雄牛や繁殖雌牛の*Q*ハプロタイプの保有状況を調べたところ、*Q*ハプロタイプは祖父牛に由来することがわかった。さらに、祖父牛の息牛で*Q*ハプロタイプを持つ別の種雄牛でも、このハプロタイプはBMSに同様の効果を持つことが示された。

（第７回日本動物遺伝育種学会大会、2006年11月、鹿児島）

学会発表11.

題　目：連鎖解析による第２のウシ乳房炎抵抗性遺伝子の同定とその機能解析

発表者：杉本真由美[1]、杉本喜憲[2]

所　属：[1]家畜改良セ、[2]畜技協・動物遺伝研

要　旨：【目的】ウシ乳房への常在細菌感染で生じる乳房炎は、国内のホルスタイン種の20-30％に発生する。乳房炎に罹ると、生産乳の出荷停止や治療費の負担だけでなく、完治は困難なため感染牛の廃棄と新規更新の経費がかかり、全国で年間690億円の被害をもたらしている。年間3000億円と云われる牛乳生産高と比べ、その負担は大きい。現在、集団選抜法により乳牛における乳生産性の遺伝的能力は充分改良されているが、生産経費を下げるためには、乳房炎抵抗性を遺伝的に高めることが急務である。そこで、連鎖解析を用いた抵抗性遺伝子の同定を行った。

【方法と結果】祖父牛を共有する６頭の種雄牛の半姉妹家系を用いて、297頭の抵抗型ウシと181頭の感受型ウシを選択タイピングすることにより、乳房炎抵抗性遺伝子候補を２つ同定した。第１の乳房炎抵抗性遺伝子は、神経発達に関与する転写因子と知られているforebrain embryonic zinc finger-like（FEZL）遺伝子であった。感受型ウシでは、FEZL遺伝子の12グリシン鎖に３塩基の挿入が見られ、13グリシン鎖に変異することにより、tumor necrosis factor α（TNFα）やinterleukin（IL）８等の免疫関連遺伝子の発現誘導の程度が低下していた。第２の乳房炎抵抗性遺伝子を同定する過程において、我々はFEZL遺伝子が当該遺伝子の発現調節に関与することを見いだした。FEZL遺伝子を過剰発現させた乳腺細胞のMicroarray解析、real-time PCR及びluciferase解析により、insulin-like growth factor 1 receptor（IGF1R)が最も有力な第２の乳房炎抵抗性遺伝子候補であった。感受型ウシは、13グリシン鎖であるFEZL遺伝子とプロモーター領域におけるシトシン鎖が長いIGF1R遺伝子を持ち、IGF1R遺伝子の発現量が抵抗型ウシより高いことが確認されたのである。IGF1R遺伝子の発現を抑えた線虫は細菌感染に強いことが既に報告されており、ウシにおいてもIGF1R遺伝子の発現が高くなると乳房炎に罹りやすいのかもしれない。我々の結果は、乳房炎抵抗性を遺伝的に高める新しい方法を提供し、FEZL及びIGF1Rは自然免疫応答にも関与していることを示唆している。

（日本免疫学会第36回学術集会、2006年12月、大阪）

学会発表12.

題　目：Linkage mapping of locus responsible for Forelimb-girdle Muscular Anomaly of Japanese black cattle on BTA 26

発表者：[1]Masoudi Ali Akbar、[2]横内　耕、[3]内田和幸、[4]大和田孝二、[1]Abdol Rahim Abbasi、[1]辻　岳人、[2]杉本喜憲、[1]国枝哲夫

所　属：[1]岡山大院自然科学、[2]畜技協・動物遺伝研、[3]宮崎大学農、[4]NOSAI宮崎

要　旨：前肢帯筋異常症は黒毛和種の常染色体上劣性の遺伝病であり、肩の異常な形態と震えを症状とする。病理所見からは、前肢帯の筋群に形成不全が見られた。我々はこの疾病の原因遺伝子を見つける目的で、連鎖解析による候補領域のマッピングを行った。同一地域の黒毛和種集団から、父方半きょうだい家系（３頭の種雄牛を含む）に属する26頭の発症牛を集め、DNAを調製した。全染色体上に配置した258のマイクロサテライトマーカーを用いて発症との関連を

調べたところ、26番染色体のテロメア側にあるマーカー群が発症と有意に連鎖していることがわかった。そこで、この領域にさらにマーカーを追加しハプロタイプを検討した結果、候補領域を10cMにまで絞り込むことができた。ヒトあるいはマウスとの比較地図から、この領域にはN-RAPやPDZ8など原因遺伝子となりうる複数の候補遺伝子が含まれている。
（第107回日本畜産学会大会、2007年３月、東京）

3．委員会、会議等の開催

1）肉用牛ゲノム研究・開発委員会

この委員会は、動物遺伝研究所が行う肉用牛のゲノム研究、開発事業のあるべき方向並びに研究開発成果の応用方向などについて審議し、必要な助言をいただくものとして開催されている。

平成18年度の委員会は平成19年２月22日東京で開催された。議事内容は次の通りであった。

① 「ウシゲノム有効活用促進事業（肉用牛DNA育種実証事業）」の概要
② 動物遺伝研究所における平成18年度研究成果と次年度事業の実施について
③ 話題提供「イネゲノムベンチャーの活動」
④ 全体討議

これらの議事の中で、動物遺伝研究所の18年度の活動の概要が資料に基づいて紹介された。研究成果並びに活動方向については諒とされた。

肉用牛ゲノム研究・開発推進委員会委員

木下　良智	（独）家畜改良センター理事長
国枝　哲夫	岡山大学農学部総合農業科学科教授
小島　信男	全国畜産関係場所長会会長
柴田　正貴	（独）農業・食品産業技術総合研究機構理事（畜産草地研究所長）
新山　正隆	（社）家畜改良事業団専務理事
菱沼　毅	（独）農畜産業振興機構副理事長
福原　利一	（社）全国和牛登録協会会長
藤山　秋佐夫	情報・システム研究機構国立情報学研究所教授

研究情勢報告者

美濃部　侑三	（株）植物ゲノムセンター代表取締役社長

2）肉用牛ゲノム研究・開発技術推進委員会

動物遺伝研究所が行う研究開発について、研究手法など技術的側面から審議し、助言をいただくとともに、研究開発成果の学術的評価もいただくものとして平成13年度よりこの委員会は設置されている。平成18年度委員会は、平成19年２月６日動物遺伝研究所で開催された。議事は次の通りであった。

① 先天性乳頭欠損症領域-1（*ATPP-1*）に存在する候補遺伝子の探索
② 経済形質遺伝子座
(a) 黒毛和種の脂肪交雑遺伝子QTL（*Marbling-3*）のファインマッピング
(b) 黒毛和種の脂肪交雑遺伝子QTL（*Marbling-1*）の責任遺伝子の同定に向けて

(c) 黒毛和種の枝肉重量遺伝子QTL（*CW-1*）の責任遺伝子の同定に向けて
(d) ゲノムワイド相関解析の現況、および、枝肉重量QTL（CW-2）領域の品種間ハプロタイプ比較
③ 平成18年度のその他の研究成果と平成19年度の研究計画について
④ 動物遺伝研究所の研究推進に対する評価及び助言について

肉用牛ゲノム研究・開発技術推進委員会委員

猪子　英俊	東海大学医学部分子生命学科教授
居在家義昭	岩手大学農学部獣医学科教授
菅野　純夫	東京大学大学院新領域創成科学研究科教授
野村　哲郎	京都産業大学工学部生物工学科教授
美濃部　侑三	（株）植物ゲノムセンター代表取締役社長

3）技術推進委員会

この委員会は、平成18年度から動物遺伝研究所が行う「牛有用性ゲノム等探索・知的財産化事業」のあるべき方向並びに研究開発成果の応用方向などについて審議し、必要な助言をいただくものとして開催されている。

第１回技術推進委員会は平成18年10月11日に東京で開催された。議事は次の通りであった。
① 畜産における知的財産化をめぐる動きについて
② 牛有用性ゲノム等探索・知的財産化事業の推進について
(a) 牛有用性ゲノム等探索・知的財産化事業の目的と概要について
(b) 事業達成目標について
③ 牛有用ゲノム、特に、牛の繁殖関連遺伝子、脂肪代謝関連遺伝子の探索について
④ 討議

第２回技術推進委員会は平成19年１月30日に東京で開催された。議事は次の通りであった。
① 牛有用性ゲノム等探索・知的財産化事業の進捗状況について
(a) 肉用牛の脂肪交雑、　SNP-DNAチップによる相関解析
(b) 枝肉重量、繁殖性、ミルク生産性、乳房炎抵抗性等
② 当該事業の平成19年度計画について
③ 討議
(a) 進捗状況（達成度）について
(b) 解析実施手法について

技術推進委員会委員

塩谷　康生	（独）農業・食品産業技術総合研究機構草地畜産研究所研究管理監
菅野　純夫	東京大学大学院新領域創成科学研究科教授
畳　　伸吾	道南NOSAI北部支所家畜診療センター支所長
新山　正隆	（社）家畜改良事業団専務理事
向井　文雄	神戸大学農学部教授

安江　博　　（独）農業生物資源研究所ゲノム研究グループ上席研究員

4）全国DNA育種推進会議

この会議は畜産新技術実用化対策事業の一環である「DNA育種基盤の確立」にかかる全国推進会議である。平成13年度からは動物遺伝研究所と道県の研究機関との共同研究はこの事業の枠組みの中で実施されることになったもので、平成12年度までの連絡調整会議に相当する。平成18年度における共同研究参画機関は21の道県（北海道、青森、岩手、宮城、秋田、山形、福島、茨城、岐阜、兵庫、鳥取、島根、岡山、広島、佐賀、長崎、熊本、大分、宮崎、鹿児島、沖縄）であり、本事業の枠組み外で動物遺伝研究所と共同研究を行っている（独）家畜改良センター、（社）家畜改良事業団も本推進会議に参加した。

第１回の平成18年度全国DNA育種推進会議は平成18年10月３日動物遺伝研究所で開催された。主要議題は次の通りであった。

① 動物遺伝研究所の新規事業について
(a) 肉用牛DNA育種実証事業
(b) 牛有用性ゲノム等探索・知的財産化事業
② 平成18年度事業の実施計画及び実施状況について
(a) 共同研究参加道県試験計画
(b)（独）家畜改良センター
(c)（社）家畜改良事業団
③ 動物遺伝研究所からの話題提供
(a) 連鎖不平衡（LD）を利用したQTLマッピング手法
(b) 半兄弟家系QTL解析のまとめーハプロタイプ比較の続き
(c) *Marbling-3*のLDマッピング
(d) *Marbling-1*、*Marbling-2*、*CW-1*の進捗状況
④ 事業に関わる特許等知的財産権の利・活用について

第２回の平成18年度全国DNA育種推進会議は平成19年２月27日東京で開催された。主要議題は次の通りであった。

① 道県共同研究機関による平成18年度の事業成果の報告
② 動物遺伝研究所の進捗状況報告
(a) 黒毛和種脂肪交雑QTL（*Marbling-1*）の責任遺伝子同定に向けて
(b) 黒毛和種枝肉重量QTL（*CW-1*）の責任遺伝子の同定に向けて
(c) 枝肉重量QTL（*CW-2*）の品種間ハプロタイプ比較によるfine-mapping
(d) ゲノムワイド相関解析の現況
③ 事業成果のまとめと今後の進め方について
「同一種雄牛の後継種雄牛家系を合わせた解析について」
④ 新規事業、和牛有用遺伝子の解明促進（知的財産活用推進）について

５）研究会等の開催

① 第１回家畜DNA西郷シンポジウムの開催

牛ゲノム解析の進展に伴い、フィールドにおけるDNA育種効果、責任遺伝子の機能解明に向けた取り組みが重要な課題となってきた。そのため内外の関係する専門家を招待し、（独）家畜改良センターと共同で、家畜DNA西郷シンポジウムを平成18年10月２日に開催した。

開催場所：（独）家畜改良センター講堂
主催：（独）家畜改良センター、（社）畜産技術協会

第１部 遺伝情報の家畜育種への応用
(1) QTL情報を利用した育種改良の可能性と今後の課題（佐藤正寛、（独）農業生物資源研究所）
(2) 遺伝情報の育種への利用効率（武田尚人、（独）農業・食品産業技術総合研究機構北海道農業研究センター）

第２部　遺伝子発現の“場”と制御機構
(3) 生殖生理におけるプロスタノイドとその受容体の役割（杉本幸彦、京都大学大学院薬学研究科）
(4) タイトジャンクションによる上皮バリア機能の制御とその異常による病態（古瀬幹夫、神戸大学医学部）

② 招待講演

動物遺伝研究所では、ウシゲノム研究の進展を図るため、平成18年度において、次のような研究者を招待し、動物遺伝研究所においてセミナーを開催した。

(1)「cDNA大量解析から見えてきた新しいゲノム像」、菅野純夫（東京大学大学院）、平成18年４月28日

(2)「ウシ脂肪交雑責任遺伝子の探索-RNA発現量を基に選抜した遺伝子のSNPと育種価との相関解析」、西村翔太（京都大学大学院）、平成18年５月１日

(3) ”Callipyge and Texel: functional analyses of microRNAs”、竹田晴子（ベルギー・リェージュ大学）、平成18年６月27日

(4) “Polymoprhic miRNA-target interactions: a novel source of phenotypic variation.”、Michel Georges（ベルギー・リェージュ大学）、平成18年11月28日

③ 動物遺伝育種シンポジウム組織委員会による第12回シンポジウム「動物ゲノム解析と新たな育種戦略：食の安全・安心を目指す分子遺伝学からのアプローチ」を動物遺伝育種学会と共催した。

開催日：平成18年11月19日
開催場所：鹿児島大学共通教育棟（鹿児島市）

内容：
セッションI.
(1) 牛肉の偽装表示を防ぐDNA品種鑑定技術（万年英之、神戸大学大学院自然科学研究科）
(2) ブタ畜産の最近の動向（安江　博、(独）農業生物資源研究所）

セッションII.
(3) 地域特産鶏肉のDNA識別の現状と課題（高橋秀彰、(独）農業・食品産業技術総合研究機構畜産草地研究所）
(4) 魚介類の原産地判別技術（山下倫明、(独）水産総合研究センター中央水産研究所）

④ 家畜ゲノム国際ワークショップ
(独）農業生物資源研究所、(社）農林水産先端技術産業振興センターと共催で家畜ゲノム国際ワークショップ「家畜ゲノム情報の育種技術への活用と新需要創出、生活の豊かさの向上に資する動物ゲノムインダストリー」を開催した。

開催日：平成18年11月９日
開催場所：日本自転車会館ホール、東京

内容：
第１部　家畜ゲノム解読の現状と今後の課題
(1) ワークショップのねらいと全体構成（粟田　崇、(独）農業生物資源研究所）
(2) 国際コンソーシアムによるブタ全ゲノム解読（Jonathan E. Beever, University of Illinois）
(3) 日本におけるブタゲノム研究の現状と今後の展望（上西博英、(独）農業生物資源研究所）
(4) 動物における比較ゲノム研究の現状と今後の展開（藤山秋佐夫、情報・システム研究機構国立情報学研究）

第２部　家畜ゲノム情報の利活用
(5) ゲノム情報を活用したブタの抗病性育種（鈴木啓一，東北大学大学院）
(6) ブタ肉質のDNAマーカーアシスト導入研究（井手華子、静岡県中小家畜試験場）
(7) ウシ肉質のDNA育種研究の現状と今後の展開（万年英之、神戸大学大学院）
(8) ブタ椎骨数に関与するQTLの単離とマーカーアシスト選抜への利用（美川　智、(独）農業生物資源研究所）
(9) ヒトのモデルとしての家畜の活用と研究展開（門松健治、名古屋大学大学院）

４．委託研究

動物遺伝研究所の研究と深く関わりを持つテーマについて、平成18年度は次の５課題を研究及び事業委託した。

1）ウシ筋肉内脂肪細胞株を用いた脂肪交雑関連遺伝子発現機構の解明（平成13年度より継続）
①委託先：東北大学大学院農学研究科
②委託研究者：麻生　久

2）黒毛和種種雄牛の多段階選抜におけるmajor genes情報の選抜指標としての効果（平成16年度より継続）
①委託先：神戸大学農学部
②委託研究者：向井文雄

3）子牛の発育不全症に係わる遺伝的研究（平成17年度より継続）
①委託先：北里大学獣医畜産学部　大動物外科学研究室
②委託研究者：渡辺大作

4）ウシとブタ、ヒト、マウスにおける遺伝子転写領域の比較（平成15年度より継続）
①委託先：佐賀大学農学部
②委託研究者：和田康彦

5）ウシ遺伝子発現解析のための、in situハイブリダイゼーション及びマイクロアレイに適したプローブの作成とそれを利用した解析（平成17年度より継続）
①委託先：（独）農業生物資源研究所ゲノム研究グループ
②委託研究者：安江　博

5．研修員の受け入れ

所　属　機　関　名	氏　　名	受け入れ期間
宮城県畜産試験場	千　葉　和　義	平18．4．19～4．28
岡山大学大学院自然科学研究科 （イラン人、大学院生）	マスーディ アリアクバル	平18．5．23～6．28
青森県農林総合研究センター	木　村　　勉	平18．6．6～6．9
京都大学大学院農学研究科（助教授）	山　田　宣　永	平18．6．26～6．30
大分県農林水産研究センター	渡　邉　直　人	平18．8．31～9．22
長崎県肉用牛改良センター	丸　田　俊　治	平18．9．14～10．6
岩手県農業研究センター	佐　藤　洋　一	平18．9．19～9．22
鳥取県畜産試験場	小　江　敏　明	平18．10．2～10．27
岡山県総合畜産センター	中　藤　由　紀	平18．10．4～10．17
茨城県畜産センター	藤　森　祐　紀	平18．10．10～10．13
沖縄県畜産センター	砂　川　隆　治	平18．10．23～10．27
宮崎県畜産試験場	井　上　和　也	平18．10．30～11．10
熊本県農業研究センター	古　田　雅　子	平18．11．6～11．17
島根県畜産技術センター	中　村　亮　一	平18．12．4～12．18
佐賀県畜産試験場	片　淵　直　人	平18．12．8～12．22
茨城県畜産センター	藤　森　祐　紀	平18．12．11～12．15
山形県農業総合研究センター	中　嶋　宏　明	平18．12．25～12．28
茨城県畜産センター	藤　森　祐　紀	平19．1．19～2．2
兵庫県立農林水産技術総合ｾﾝﾀｰ	秋　山　敬　孝	平19．1．22～1．26
大分県農林水産研究センター	渡　邉　直　人	平19．1．22～3．2
佐賀県畜産試験場	片　淵　直　人	平19．2．5～2．16
熊本県農業研究センター	古　田　雅　子	平19．2．5～2．16
沖縄県畜産研究センター	砂　川　隆　治	平19．2．7～2．21
宮城県畜産試験場	千　葉　和　義	平19．2．8～2．9
青森県農林総合研究センター	木　村　　勉 鈴　木　晶　夫	平19．2．13～2．22
鹿児島県肉用牛改良研究所	瀬戸口　浩　二	平19．2．19～2．27
島根県畜産技術センター	中　村　亮　一	平19．3．7～3．20
岐阜県畜産研究所	小　林　直　彦	平19．3．12～3．16

6．職員の普及活動等

1）講演

1. 杉本喜憲：DNA解析研究に関する講演、黒毛和種の経済形質解析。平成18年度DNA解析に関する検討会、岐阜県畜産研究所、2006年10月、高山。

2. 渡邊敏夫：「ハプロタイプを用いた牛肉の品種鑑定」、JRA畜産振興事業に関する調査研究発表会、2006年11月、東京。

3. 杉本喜憲：「黒毛和種牛における経済形質遺伝子（霜降り遺伝子と発育増進遺伝子）の探索」、JRA畜産振興事業に関する調査研究発表会、2006年11月、東京。

4. 平野　貴：「黒毛和種大規模家系QTL解析の現状と展望」、平成18年度DNA解析に関する検討会、岐阜県畜産研究所、2007年１月、高山。

5. 杉本喜憲：「家畜ＤＮＡ研究の成果と現状」、（社）家畜改良事業団家畜改良技術研究所、2007年３月、前橋。

6. 杉本喜憲：「家畜ＤＮＡ研究の成果と現状、そして未来」、2007年３月、別府。

7. 杉本喜憲：「家畜ＤＮＡ研究の現状」、2007年３月、大分県農林水産研究センター畜産試験場、竹田。

第３節　総　　務

1．職員名簿

（平成19年３月31日現在）

所　　属	職　　名	氏　　名
所　　長	所　　長	杉　本　喜　憲
管　理　部	部　　長	高　田　耕　節
	補　助　員	浅　比　紀　子
動物遺伝研究部	部　　長	高須賀　晶　子
	主任研究員	渡　邊　敏　夫
	研　究　員	溝　口　　康
	研　究　員	平　野　　貴
	研　究　員	横　内　　耕
	研　究　員	高　野　　淳
	補　助　員	渡　辺　恵美子
	補　助　員	塚　澤　浩　子
	補　助　員	藤　井　友　子
	補　助　員	鳴　島　亜希子
	補　助　員	金　内　由美子
	補　助　員	丸　山　久美子
	補　助　員	真　船　文　恵
	臨時職員	西　村　翔　太

2．職員の異動

1）職員の採用

採用年月日	氏　　名	所　　属	備　　考
平成19年３月12日	西　村　翔　太	動物遺伝研究部臨時職員	

2）職員の退職・退任

退職・退任年月日	氏　　名	所　　属	備　　考
平成19年２月28日	相　馬　千　裕	動物遺伝研究部補助員	
平成19年３月31日	星　　優　美	動物遺伝研究部補助員	

3．職員の海外出張

氏　名	出張先	期　間	用　　務
渡　邊　敏　夫	ブラジル	18.8.11 - 18.8.18	ブラジル国ベロオリゾンテ市で開催された第８回世界家畜生産応用遺伝学会議に参加
渡　邊　敏　夫	ブラジル	18.8.19 - 18.8.28	ブラジル国ポルトセグロ市で開催された第30回国際動物遺伝学会2006年大会に参加（上記遺伝学会に引き続き旅程）
高須賀　晶　子	ブラジル	18.8.18 - 18.8.28	ブラジル国ポルトセグロ市で開催された第30回国際動物遺伝学会2006年大会に参加
横　内　　耕	ブラジル	18.8.18 - 18.8.28	ブラジル国ポルトセグロ市で開催された第30回国際動物遺伝学会2006年大会に参加
高須賀　晶　子	アメリカ	19.1.12 - 19.1.19	米国サンディェゴ市で開催された第15回植物・動物ゲノム学会に参加

4．施設・機器の整備

1）施　設

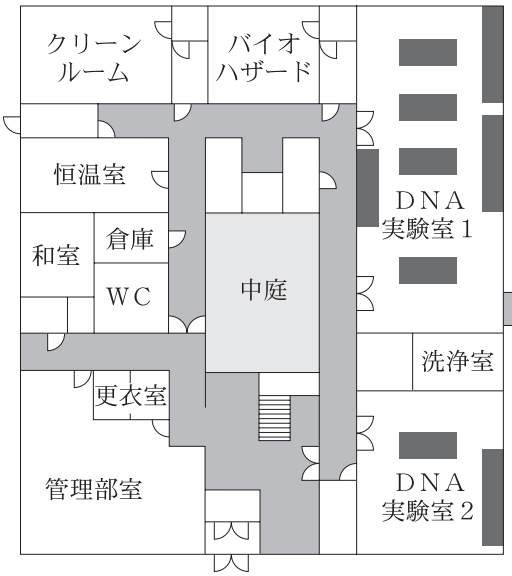
研究施設の平面図は31ページの通り。

2）平成18年度購入の主要機器（単価百万円以上）

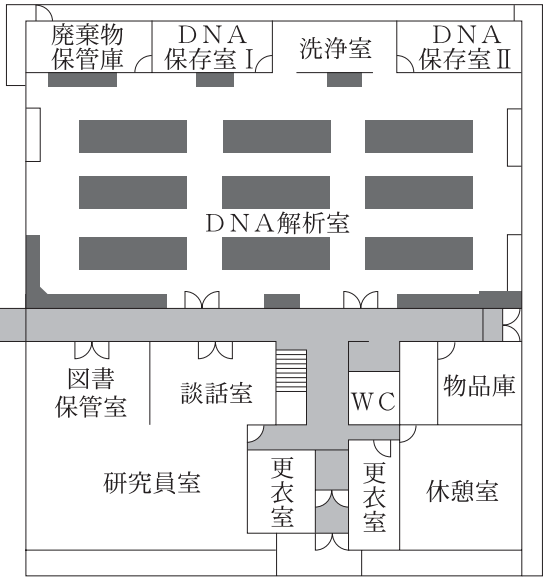
機　　器　　名	式　　数
DNAシーケンサー（Applied Biosystems 3730xL-20）	1
ルミノメーター（プロメガ社製　96ウエルプレート対応）	1

研 究 施 設 平 面 図

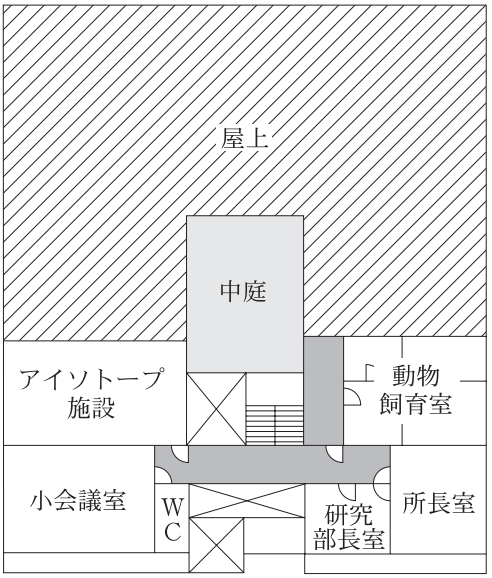
本館１階（742㎡）



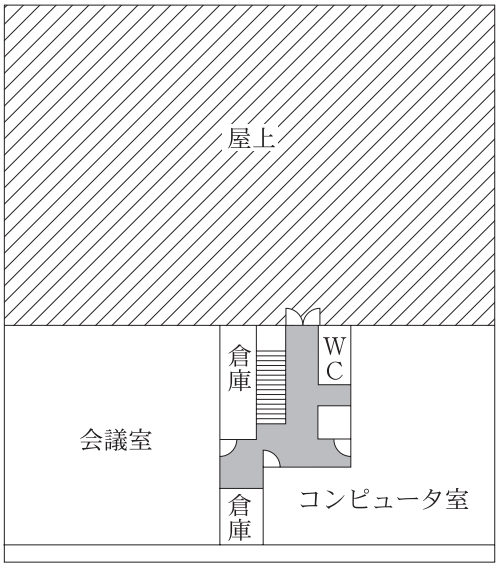
別館１階（788㎡）



本館２階（258㎡）



別館２階（306㎡）



第４節　資　　料

5．購読雑誌一覧

1）Animal Genetics
2）Cell
3）Cell Metabolism
4）Current Biology
5）Genome Research
6）Genomics
7）Genes & Development
8）Journal of Biological Chemistry
9）Mammalian Genome
10）Molecular Cell
11）Nature
12）Nature Genetics
13）Nature Medicne
14）Nature Reviews Genetics
15）Nature Reviews Molecura Cell Biology
16）Science
17）The American Journal of Human Genetics
18）The Journal of Cell Biology
19）Trends in Genetics
20）細胞工学
21）実験医学
22）畜産技術

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High-resolution physical mapping and construction of a porcine contig spanning the intramuscular fat content QTL

S. Sato*,†, H. Hasebe*, S. Sato*, Y. Asahi*, T. Hayashi†, E. Kobayashi* and Y. Sugimoto‡

*National Livestock Breeding Center, Nishigo, Fukushima 961-8511, Japan. †National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-0901, Japan. ‡Shirakawa Institute of Animal Genetics, Nishigo, Fukushima 961-8061, Japan

Summary

We previously mapped a locus for porcine intramuscular fat content (IMF) by linkage analysis to a 17.1-cM chromosome interval on *Sus scrofa* chromosome 7 (SSC7) flanked by microsatellite markers *SW1083* and *SW581*. In this study, we identified 34 microsatellite markers and 14 STSs from the 17.1-cM IMF quantitative trait loci (QTL) region corresponding to HSA14q and aligned those loci using the INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel. We then constructed a 5.2-Mb porcine bacterial artificial chromosome (BAC) contig of this region that was aligned using the RH panel. Finally, the IMF QTL was fine-mapped to 12.6 cM between *SJ169* and *MM70* at the 0.1% chromosome-wise significance level by genotyping the previously studied F_2 resource family with 17 additional microsatellites. We also demonstrated that the *SJ169–MM70* interval spans approximately 3.0 Mb and contains at least 12 genes: *GALC*, *GPR65*, *KCNK10*, *SPATA7*, *PTPN21*, *FLJ11806*, *EML5*, *TTC8*, *CHES1*, *CAP2P1*, *CHORDC2P* and *C14orf143*.

Keywords bacterial artificial chromosome contig, gene, intramuscular fat content, *Sus scrofa*.

Introduction

Intramuscular fat content (IMF) is one of the most economically important traits affecting meat quality in pigs. However, there are some studies that do not find a significant positive correlation between IMF and meat quality, and an excess of visible fatness can reduce consumer acceptability of fresh meat and cured cooked hams. Consequently, increasing the level of IMF without increasing the level of visible fat could be a way to improve the acceptability of fresh pork. An optimum IMF content of approximately 2.5–3.5% has been suggested (Fernandez *et al.* 1999a,b, 2000). Improvement of IMF by selective breeding is often difficult and expensive because of measurement difficulties, but marker-assisted selection may improve accuracy in evaluation of IMF for young animals (Meuwissen & Goddard 1996).

Address for correspondence

Shuji Sato, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-0901, Japan
E-mail: sshuji@affrc.go.jp

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Janss *et al.* (1997) initially found evidence for major genes affecting meat quality traits, including IMF, and over the past several years, several studies have reported quantitative trait loci (QTL) for IMF. de Koning *et al.* (1999) reported IMF QTL on *Sus scrofa* chromosome 2 (SSC2), SSC4 and SSC6 using F_2 animals from a Meishan-by-Dutch Large White cross, whereas their half-sib analysis showed suggestive linkage of IMF on SSC4 and SSC7. Harlizius *et al.* (2000) reported IMF QTL on the X chromosome at the 5% genome-wise level using the same F_2 population. Malek *et al.* (2001) reported QTL for marbling on SSC1, SSC8 and SSC10, one of which was significant at the 5% genome-wise level using crosses between Berkshire and Yorkshire commercial breeds. We identified IMF QTL on SSC7, SSC9 and SSC13 using a porcine F_2 resource population produced from a Meishan sow and a Duroc boar (Sato *et al.* 2003). The IMF QTL on SSC7 was detected at the 1% genome-wise level, and its position was similar to the result of de Koning *et al.* (1999) using the half-sib analysis.

Methods for identifying a responsible gene by positional cloning include the construction of a bacterial artificial chromosome (BAC) contig for the QTL region. This study reports the construction of a 5.2-Mb BAC contig for SSC7

that includes 54 microsatellite markers, 13 genes and 124 sequence-tagged sites (STS).

Materials and methods

F_2 resource population and marker genotyping

We used a resource population comprising 865 F_2 animals produced from the cross of a Meishan sow and a Duroc boar (Sato *et al.* 2003). One-hundred and sixty-five males were measured for IMF content and used to localize the IMF QTL on a linkage map. Primer pairs used for polymerase chain reaction (PCR) amplification of the microsatellite markers are reported in Table 1. Genotyping was performed using standard procedures as described in Sato *et al.* (2003).

Map construction and QTL mapping

Although a linkage map had been obtained by Sato *et al.* (2003), a new linkage map including 17 additional microsatellite markers developed in the IMF QTL region (Table 2) was constructed for SSC7 using CRI-MAP 2.4 software (Green *et al.* 1990). Based on this new linkage map of SSC7, we reanalysed QTL for IMF using the method developed by Haley *et al.* (1994). The *F* value was used as a test statistic for detecting QTL, and the chromosome-wise threshold was obtained by permutation (Churchill & Doerge 1994) of 5000 repetitions. A threshold of *F*-value at the 0.1% chromosome-wise significance level was 11.27.

RH mapping

The 7000-rad INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel comprising 118 hybrid clones (Yerle *et al.* 1998) was used to generate a RH map for the IMF QTL region. Genotyping of individual clones was performed under standard PCR conditions. The PCR products were run on 2% agarose gels and visualized by ethidium bromide staining. We used the mapping system provided at http://imprh.toulouse.inra.fr.

BAC library screening

We used two porcine BAC libraries for constructing a BAC contig of the IMF QTL region. The first was a 4.4X genome equivalent porcine BAC library constructed in the pBAC-Lac vector and comprising 103 488 clones with an average insert size of 133 kb (NIAS GenBank, Ibaraki, Japan). The BAC clones from this library were screened by PCR using a four-dimensional pooling scheme (Suzuki *et al.* 2000). The second porcine BAC library contained 185 389 clones in the pTARBAC2 vector with an average insert size of 165 kb (10.2X genome coverage), which was purchased from the Children's Hospital Oakland Research Institute (RPCI-44, Oakland, CA, USA) and screened by filter hybridization. The high-density nylon filters from the library were hybridized with ^{32}P-labelled PCR products (Random Primer Labeling Kit, Stratagene, La Jolla, CA, USA) generated using standard procedures. Probes corresponded to STSs in the chromosomal region, including STSs derived from BAC end sequences. Filters were pre-hybridized for 30 min and then hybridized with labelled probes overnight at 65 °C in Rapid Hybridization Buffer (Amersham Biosciences, Uppsala, Sweden). After hybridization, the filters were washed once with wash buffer [0.1% sodium dodecyl sulphate (SDS), 2 × SSC] for 30 min at room temperature and twice with wash buffer for 30 min at 65 °C. Filters were exposed to film for approximately 2 h.

BAC end sequencing

The BAC DNA was prepared from bacterial pellets obtained from 300-ml overnight cultures started from a glycerol stock solution with 12.5 μg/ml of chloramphenicol. Plasmid DNA was extracted by a standard alkaline lysis procedure, precipitated with isopropanol, and resuspended in 150 μl of TE [10 mM Tris-HCl (pH 8), 1 mM ethylenediaminetetraacetic acid (EDTA)]. Cycle sequencing was carried out using the BigDye Terminator Kit (PE Applied Biosystems, Foster City, CA, USA), purified on Sephadex™ G-50 Fine (Amersham Biosciences) in 96-well format and analysed on an ABI 3700 capillary sequencer (PE Applied Biosystems). The reactions were performed in a final volume of 20 μl containing 8 μl ABI BigDye terminator mix and 12.5 pmol sequencing primer (M13 forward and M13 reverse, or T7 and SP6). Reactions were carried out on a PE-9600 thermocycler (PE Applied Biosystems) and cycled 35 times (96 °C for 30 s, 50 °C for 20 s and 60 °C for 4 min). Primers were designed from BAC end sequences and used for chromosome walking and gap closures.

Development of microsatellite markers

About 500 ng BAC DNA were digested with *Alu*I to completion and subcloned into a pUC118 dephosphorylated vector (Takara, Kyoto, Japan). Sublibraries plated on 15-cm Petri dishes were screened by colony hybridization with Poly(dA-dC)Poly(dG-dT) (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) that was labelled with a Random Primer Labelling Kit (Stratagene) according to standard protocols. Positive clones were sequenced to confirm the presence of microsatellites, and the sequence was used to design PCR primers for amplifying the microsatellite markers.

Results and discussion

Radiation hybrid mapping of microsatellite markers and genes

The human and mouse genomic segments corresponding to the region between *SW1083* and *SW581* on SSC7q were

Table 1 Primers used for RH mapping and gene mapping on swine chromosome 7.

Gene or marker	Primer (5′–3′; forward/reverse)	Source, reference and/or GenBank accession number	Length (bp)
ANPEP	F: aag tca gct ggc ctg tca gc R: aac tca gtg aca gac cag ttg	X16088	480
CKB	F: ggg cgg cag gag aag tct c R: gac ggg gtg aag ctg ctc a	AJ000955 AJ000956	220
DNCH1	F: agc ctc acc atc gac ttc R: ctt ccc gtc tac ctg aac tt	F14756	239
EIF5	F: tca gct tat ctc caa gat tcc R: tct tcg cca cca gaa gat tc	U49436[4]	201
TDP1	F: gac gtc agg ggg agg cc R: tga tgt gga gag ctc ctg a	BE233002	119
SERPINA	F: ctc tcc ctg ggg acc aag R: ctt ggc ctc ttc ggt gtc	X88780, Jiang *et al.* (1998)	273
PKM2	F: gcc ttc att cag acc cag ca R: att cca gac tta atc atc tcc tt	Venta *et al.* (1996)	500
RPS6KA5	F: tcc gag ttc tgg agc cgc R: gca gct cgt gct tga cag t	BI443934	220
TRIP11	F: tcc agg tgt ggt aga aga ac R: tct ctg gct tga gaa tta tct a	AW483209	229
TSHR	F: ctc cta cca aga atg gaa gt R: tta taa ggc ttg tgg gct ac	AF055631	397
YY1	F: cca aga aca ata gct tgc cc R: cca gta tga acc agt tgg tg	M76541[3]	170
FLRT2[5]	F: aca att cta cag ctt ttt ctg a R: ggc atg tgc ccc ata cat g	Porcine BAC 419A21	180
	F: gac taa aat gga aac caa agt c R: gtt tag gta taa aat gct atg c	BF709901	232
ATXN3[4]	F: cac tct ggc caa tat cta aat g R: aaa tat ggc cat gga caa ctc	Porcine BAC 37686	140
	F: caa gaa ggc tcg ctt tgt g R: ctc ctt ctg cca ttc tca tc	AW414522	130
KIAA2010[6]	F: gag aac ttc atc ata gat gta a R: aat ctt gaa atc gcc cca ga	Porcine BAC 838A9	120
	F: ggc atg gat gat aca cag gt R: cat cat cat tct gtt gtg cct	BF075675	118
KCNK10	F: aga gca tca aca acc ggc R: ttc cgt ctc ctc ctc ttt c	TC13956[2]	233
SPATA7	F: tta gca acg gcc tgg cga c R: tcc atg ctt agt cct ctt gc	AF144487[3]	153
PTPN21	F: tct ctg cca gta cac att tgt R: atg agt tag gca agc gct ttt	X79510[1]	141
FLJ11806	F: cag tga cac tta cat atg gtt R: tta gaa ggt gga aga gaa gg	AK021868[5]	800 (445 + intron)
EML5	F: tca ctg gtg tac agt cta ag R: tca aat att cac tat ttg ctc c	BQ602582	230
TTC8	F: tga aaa cga aga gga ggt ag R: ttg ttg tag gcc tcg gcg t	AW436589	139
CHES1	F: act atg agt ttg cca cca ag R: tga gtt cta tgg ctt caa tct	NM_005197[3]	605
LOC400236	F: cac ggc aag cta ctg cag a R: gga gga ggt cca ttt gca aa	AI133173[3]	139
KCNK13	F: tac ctg tcc cca ctg gtc R: gag gaa cgt ggt gat gcc	TC16625[2]	297
CALM1	F: gaa ttc aag gaa gct ttc tcc R: tac cat cag cgt cca cct c	BC17806[4]	148

Table 1 Continued

Gene or marker	Primer (5′–3′; forward/reverse)	Source, reference and/or GenBank accession number	Length (bp)
SJ111[1]	F: act tgg cat tct gta cct aa R: att cta ctg ctg acc aaa ag	AB237156	266–278
SJ169[1]	F: ttg cat ttc aca tca ctt tc R: gct cag gct cag tca tat att	AB237157	221–230
SJ300[1]	F: agg aga tcc tgc tgc att gaa R: gct tgt caa cca tgg gag agg	AB237158	299–314
SJ492[1]	F: ctc gcc cat aaa cca tag caa R: aag cgg ttg agt tga gcc aat	AB237159	142–148
SW2002	F: cac atg gtc ttt gca agc c R: gtg tgc aaa gtt cat gca gg	AF253770	106–112
SWR773	F: gtg gct ggg gta tag gcc R: tgc tga agc atc cac ttc ac	AF235492	134–141

[1]These markers were also used to fine map the IMF QTL on SSC7.
[2]These number indicated the origin from the TIGR Pig Gene Index.
[3]Human sequence.
[4]The second set of primers listed for these markers were only used for screening the corresponding BAC clones.
SSC7, *Sus scrofa* chromosome 7; QTL, quantitative trait loci; RH, radiation hybrid; BAC, bacterial artificial chromosome; IMF, intramuscular fat content.

determined by comparative mapping. Heterologous chromosome painting experiments previously identified SSC7q as orthologous to part of human chromosome 14 (HSA14; Rettenberger *et al.* 1995), and mouse chromosome 12 (MMU12) is orthologous to the same region of human HSA14 (Copeland *et al.* 1993). To align the linkage map of distal SSC7q with transcript map of distal HSA14q and MMU12, we obtained STSs from genes spanning distal HSA14q. We successfully amplified the porcine orthologues of 14 genes located on HSA14q and MMU12: *ANPEP*, *PKM2*, *SERPINA*, *DNCH1*, *CKB*, *TSHR*, *EIF5*, *YY1*, *TDP1*, *TRIP11*, *RPS6KA5*, *FLRT2*, *ATXN3* and *KIAA2010* (Table 1).

The porcine IMpRH panel (Yerle *et al.* 1998) was used for high-resolution mapping of six microsatellite markers, including *SJ111*, *SJ169*, *SJ300*, *SJ492* (AB237156–AB237159 respectively), *SW2002* and *SWR773* (Rohrer *et al.* 1996) and 14 genes. Three of the 14 genes (*FLRT2*, *ATXN3* and *KIAA2010*) were used to screen BAC libraries, and the identified BAC clones were end sequenced. The primers designed from the end sequences were used for RH mapping. For the other 11 genes, primers for RH mapping were designed from the sequences of the genes, and they did not amplify hamster DNA. Two-point analysis confirmed the predicted locations in SSC7 for the 14 genes on human HSA14, as all yielded LOD scores >3.0 with at least one of the microsatellite markers. Positions of the genes on the RH map relative to the microsatellite markers were then determined by using multipoint analysis, fixing the order of the microsatellite markers according to the linkage map. Results indicated that *ANPEP*, *PKM2* and *SERPINA* are located proximally to *SW1083*, while *ATXN3*, *TRIP11*, *RPS6KA5*, *KIAA2010*, *DNCH1*, *CKB*, *EIF5* and *YY1* are located distal to *SW581*. *TSHR*, *FLRT2* and *TDP1* are potentially located within the *SW1083*–*SW581* interval. Based on RH mapping, the following 10 markers were used as anchor points: *TSHR*, *TDP1*, *FLRT2*, *SJ492*, *SJ169*, *SJ300*, *SJ111*, *SWR773*, *SW1083* and *SW581*.

High-resolution physical mapping and QTL analysis

To elucidate further the porcine IMF QTL region on SSC7 and to confirm the gene order on the linkage and RH maps, we constructed a BAC contig for the region using 10 markers as anchor points. We first identified 10 BAC clones using these markers as probes. Additional BAC clones were subsequently identified by chromosome walking using BAC end sequences from the original 10 BAC clones. Chromosome walking was conducted in both directions until BAC clones overlapping existing clones were found. All but one of the original clones used as anchors generated one large contig consisting of 68 BAC clones. The clone containing *TSHR* remained as a BAC singleton. These BAC clones were used to identify 34 microsatellite markers (Table 2), 13 of which were polymorphic in the resource population. One-hundred and sixty-five F_2 males of the resource population (Sato *et al.* 2003) were genotyped to refine the region with 17 additional polymorphic markers including the 13 new microsatellite markers, as well as *SJ492*, *SJ169*, *SJ300* and *SJ111* between *SW1083* and *SW581* (Tables 1 and 2). The resulting genotypes were used to construct a linkage map for the IMF QTL region. The IMF QTL on SSC7 was reanalysed, and the confidence region for the IMF QTL where the *F*-value exceeded the 0.1% chromosome-wise significance level threshold was a 12.6-cM interval between *SJ169* contained in BAC 91A7, and *MM70* contained in BAC 178I8 (Fig. 1).

Table 2 Microsatellite markers developed from BACs on swine chromosome 7 in the QTL interval for intramuscular fat content.

Microsatellite	BAC clone	Primer (5′–3′) sequence	Temperature (°C)	Length (bp)	GenBank accession number
MM64	732D9	F: tgg gaa ctg taa ttc cat aaa t R: tcc ttt gtt cct tct gtg gc	58	155–157	AB206688
MM81	732D9	F: gaa agg atc tgt tga ggt ttg R: aaa tag taa cag cag aca cac	58	164–168	AB206697
MM84[1]	456F6	F: ctc cat tct ctc ctg cag ta R: ata aga agg aag tga gta gtg	58	153–163	AB206700
MM82	1035F10	F: aca aat gtg gac tca agg atg R: gga agc aaa aag atg gac cat	60	200–204	AB206698
MM88	518A5	F: gtc cat aat gat aac aga agc a R: aac cct tcg tat att agg aga a	60	284–290	AB206701
MM67	201H4	F: tgg gta agc agc cca aga aat R: cat caa tca tca gac ctg aga	60	141–148	AB206689
MM96	190D8	F: gat tcc ctc atc aaa taa agt g R: tgg gct act gca cag tat ca	60	258–277	AB206706
MM68[1]	601C12	F: cca aag tga tca caa aag aag t R: cgt gat taa tga ggg aat ggt	60	190–197	AB206690
MM90	648B7	F: caa cac aca aag caa ggc ct R: caa gga gtg tct ctc cat gt	60	155–157	AB206702
MM91	648B7	F: gtg aaa tga tcc tga gga gg R: gac cgt att atc cca gtg ag	60	242–256	AB206703
MM92	648B7	F: ccg tga atg agg ccc taa aa R: tga aca tgg aat atg ccc tca	60	218–222	AB206704
MM83[1]	189D5	F: aga cca cct gat gtt tgc ct R: atc aca tag tct cat cac aag	58	null 186	AB206699
MM93	897C7	F: tgg aaa aaa ata aaa atc act tta g R: atc tga ata ggg caa tta act g	58	157–161	AB206705
MM117[1]	917E1	F: gcc att ctt gag taa ggc tg R: ccg agt aac agg att gaa gg	60	133–157	AB206674
MM78	167D5	F: gga ttc gtt aac cac tgc g R: ggg cat ttt aat ccc cga tt	58	215–224	AB206696
MM77	167D5	F: cac ata gag agg cag cag tt R: gga gcg gcc caa gaa atc	58	263–270	AB206695
MM115[1]	795D12	F: caa act gtt gaa gtc aga atc a R: tgc cca ggt caa tct ggt g	60	166–172	AB206673
MM43[1]	850F3	F: ctg tat aga ccc acc tgc ct R: gtc agt gaa gct agc act tg	60	226–234	AB206680
MM21[1]	222G4	F: cag tag tac tct gct acg ga R: tga ttt tag tgc tga ctc agc	60	152–168	AB206678
MM22[1]	222G4	F: gtt cac ggt cat ggc cgt t R: gtc agg cca acc tgg aac t	60	107–115	AB206679
MM69	178D8	F: tct gat gga gcc aag acg at R: tgg gtt aag gat cca gga tg	60	143–159	AB206691
MM70[1]	178D8	F: tat gcc ttc ttg gga aat gaa t R: aaa ttc ctt gct ggc aag gg	60	271–276	AB206692
MM118[1]	177E12	F: gtc cag ggc ttt gct ttt ca R: aac gca aaa tcc cat tac ctg	60	176–207	AB206675
MM119[1]	177E12	F: aga gga aac caa gag agg ta R: acg cat ata cat cta cat gtc	60	165–181	AB206676
MM120[1]	177E12	F: taa tca cat gaa cct gtt tct t R: cgg atc caa acc att gcc t	58	258–267	AB206677
MM148[1]	962D8	F: gag gga ctg cag agt ttt tg R: tct tct gcc cgt ggc tca g	60	247–253	AB206682
MM145	1052H1	F: tcc atg tgc cat ggg tgc a R: ctt gat ttc ccc aat aca acc	60	195–209	AB206681
MM73	487C10	F: cag cat att tgg aaa agg cca R: ttt gtc tca gat ctc aac ctg	60	147–149	AB206693

Table 2 Continued

Microsatellite	BAC clone	Primer (5′–3′) sequence	Temperature (°C)	Length (bp)	GenBank accession number
MM75	487C10	F: tgt gtg tgg ccc taa aag g R: ccc tct ctc cat aaa tga gt	58	203–210	AB206694
MM53	648D9	F: aaa agg ctg ctt cca cat tc R: tcc ctg ggc ctc cct caa	58	126–138	AB206685
MM51	817D7	F: tca atg atg ttc agg gcg tc R: ctc aag tgg tgg ctt cgt gt	60	212–238	AB206684
MM58	886E8	F: gcc agg ata tca gga aat aat t R: tga gtt ccc ctt aaa ttc aga a	60	119–131	AB206687
MM57	614B9	F: gct aca agt cgc cag gcc R: gct ggt tgc cct ctt ctc t	60	184–186	AB206686
MM50	727B6	F: ttt cag agc aga tcc ctc g R: cac aga tgc ata tgt ata ctt a	58	232–250	AB206683

[1]These markers were also used to fine map the IMF QTL on SSC7.

QTL, quantitative trait loci; BAC, bacterial artificial chromosome; IMF, intramuscular fat content; SSC7, *Sus scrofa* chromosome 7

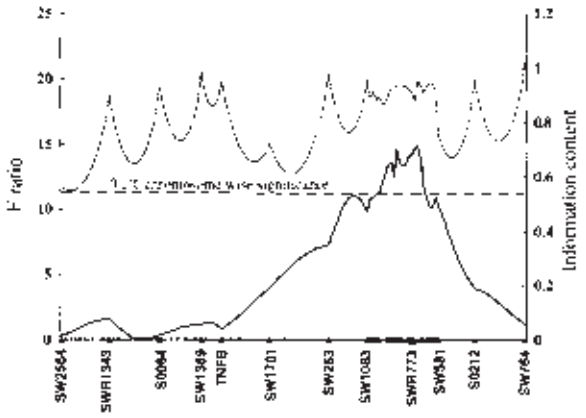

Figure 1 High-resolution linkage mapping of the intramuscular fat content (IMF) quantitative trait loci (QTL) on *Sus scrofa* chromosome 7 (SSC7). The thin line indicates information content; the bold line indicates the *F*-ratio. The threshold value for the chromosome-wise significance level of 0.1% was 11.27 (dashed line)

The BAC contig contained 68 BAC clones and spanned approximately 5.2 Mb (Fig. S1). The physical size of the *SJ169–MM70* interval was estimated at approximately 3.0 Mb based on the human draft sequence (http://www.ncbi.nlm.nih.gov/mapview; Build 35). It is likely that this region is a recombination hotspot because the genetic distance calculated from the linkage analysis was 12.6 cM. In the course of screening BAC clones, 124 STSs were determined and mapped on the contig. The average STS marker density across the contig was 1 per 42 kb of genomic sequence.

Gene order and comparative mapping

To more accurately confirm the order of the genes within BAC contig, DNA from each BAC clone was used for PCR amplification with 13 genes: *FLRT2*, *KCNK10*, *SPATA7*, *PTPN21*, *FLJ11806*, *EML5*, *TTC8*, *CHES1*, *LOC400236*, *TDP1*, *KCNK13*, *CALM1* and *RPS6KA5*. These primers were developed from pig expressed sequence tags (ESTs) or from human ESTs spanning distal HSA14q (Table 1) based on Map Viewer of the National Center for Biotechnology Information (NCBI) database. Results from these PCRs indicate that the most likely positions of *KCNK10*, *SPATA7*, *PTPN21*, *FLJ11806*, *EML5*, *TTC8*, *CHES1* and *LOC400236* were within the *SJ169–MM70* interval while *TDP1*, *KCNK13*, *CALM1* and *RPS6KA5* could be excluded (Fig. S1). However, the possibility that the responsible gene is located outside of the defined interval was not excluded. The IMF QTL region in the porcine genome corresponds to human HSA14q22–q24 and mouse MMU12 (Fig. 2). Comparison of the region with human gene map indicated that 12 genes should be located in this critical interval: *GALC*, *GPR65*, *KCNK10*, *SPATA7*, *PTPN21*, *FLJ11806*, *EML5*, *TTC8*, *CHES1*, *CAP2P1*, *CHORDC2P* and *C14orf143*. However, there is no obvious relationship between these genes and the IMF phenotype or adipogenesis. Seven phenotypes have been mapped by linkage to the HSA14q24–14q31 interval. Five of these phenotypes are probably not associated with IMF phenotype. However, two diabetic phenotypes have been mapped to this region. Field *et al.* (1996) attributed susceptibility to insulin-dependent diabetes mellitus (IDDM1) to a locus on human chromosome 14q24.3–q31. Kim *et al.* (2003) reported that mutations in hepatocyte nuclear factor 1-α accounted for the largest proportion of maturity onset diabetes of the young (MODY) cases in the USA, and this phenotype was mapped on human chromosomes 5p15, 9q22 and 14q24. Further efforts for positional cloning of the IMF QTL on SSC7 are needed, including an analysis of linkage disequilibrium using a random sample with IMF phenotypes, because this critical region cannot be refined using our resource population.

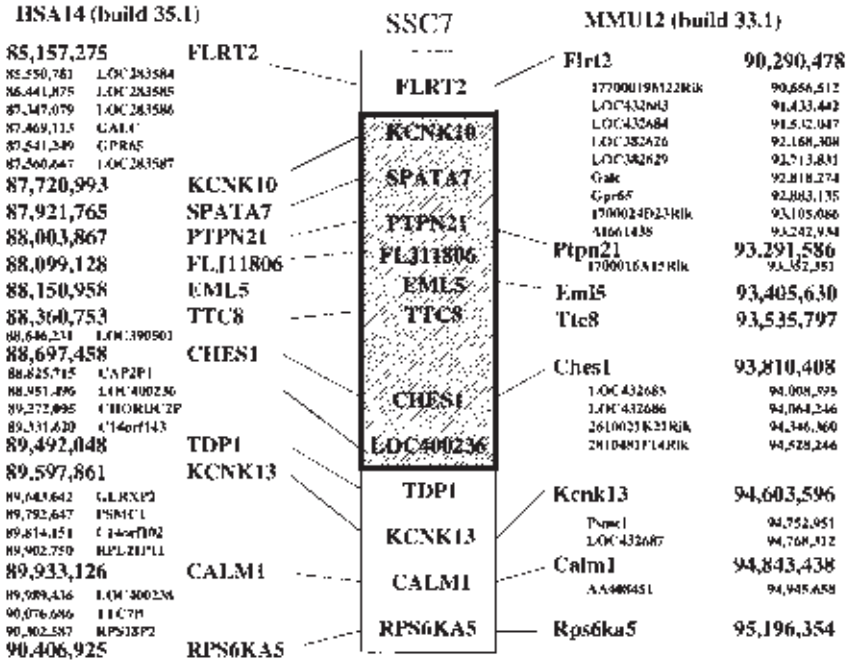

Figure 2 Comparative map for *Sus scrofa* chromosome 7 (SSC7), HSA14 and MMU12. Human and mouse gene position data were from NCBI (http://www.ncbi.nlm.nih.gov). The shaded box was the 0.1% chromosome-wise quantitative trait loci (QTL) region.

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Supplementary material

This following material is available as part of the online article from http://www.blackwell-synergy.com:

Figure S1. BAC contig of the IMF QTL on *SSC7*.

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Genome-wide linkage disequilibrium in two Japanese beef cattle breeds

M. Odani*, A. Narita*, T. Watanabe†, K. Yokouchi‡, Y. Sugimoto‡, T. Fujita‡, T. Oguni§, M. Matsumoto§ and Y. Sasaki*

*Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture, Kyoto University, Sakyo, Kyoto, Japan †Shirakawa Institute of Animal Genetics, Nishigo, Fukushima, Japan. ‡Oita Prefectural Institute of Animal Industry, Kuju, Oita, Japan. §Kumamoto Agricultural Research Center, Goushi, Kumamoto, Japan

Summary

There is little knowledge about the degree of linkage disequilibrium (LD) in beef cattle. This study aims to perform a genome-wide search for LD in Japanese Black and Japanese Brown beef cattle and to compare the level of LD between these two breeds. Parameter D' (the LD coefficient) was used as a measure of LD, and LD was tested for significance of allelic associations between syntenic and between non-syntenic marker pairs. Effects of breed, chromosome, genetic map distance and their interactions with D' were tested based on least squares analyses. Both breeds showed high levels of LD, which ranged over several tens of cM and declined as the marker distance increased for syntenic marker pairs. A rapid decline of the D' value was observed between markers that were spaced 5 and 20 cM apart. LD was significant in most cases for marker pairs <40 cM apart but was not significant between non-syntenic loci. The pattern of LD found in these two breeds was similar to that previously published for dairy cattle. The D' value between breeds was not significantly different ($P > 0.05$), but the interaction between breed and chromosome was highly significant ($P < 0.0001$). Genetic selection seems to have caused the heterogeneity of the D' values among chromosomes within breed. These results indicate that LD mapping is a useful tool for fine-mapping quantitative trait loci of economically important traits in Japanese beef cattle.

Keywords beef cattle, fine-mapping, linkage disequilibrium, selection.

Introduction

In recent years, the number of genetic markers linked to quantitative trait loci (QTL) of economically important traits has dramatically increased in livestock (e.g. Andersson 2001). After detection of the QTL-linked markers, two important issues need to be resolved: (i) the identification of causal genes controlling the traits; and (ii) the identification of QTL-linked markers for marker-assisted selection (MAS) to improve the accuracy of genetic evaluation (Fernando & Grossman 1989). To date, at least two excellent experiments have produced convincing evidence of causal mutations for QTL or quantitative trait nucleotides in livestock (Van Laere *et al.* 2003; Grisart *et al.* 2004).

Address for correspondence

Yoshiyuki Sasaki, Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
E-mail: sasaki@kais.kyoto-u.ac.jp

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Linkage disequilibrium (LD) is a non-random association of alleles at different genetic loci in a population. LD has received much attention as it provides a potential way of fine mapping a QTL region underlying a trait (Terwilliger & Weiss 1998). By making full use of historical recombination events, LD mapping has the potential to position QTL to a small chromosomal segment, perhaps on the order of <1 cM (Ardlie *et al.* 2002), and it has been used to attain mapping resolution down to the sub-cM level (Van Laere *et al.* 2003; Grisart *et al.* 2004). The usefulness of LD in fine-mapping and MAS depends on the degree of LD, the distribution and heterogeneity of LD across the genome and its relationship with genetic map or physical distances in the population.

Previous studies on genome-wide LD in livestock species have shown that considerable LD spans large genetic distances (>20 cM) in Dutch dairy cattle (Farnir *et al.* 2000), New Zealand sheep (McRae *et al.* 2002) and canine (Lindblad-Toh *et al.* 2005) populations. These studies have shown significant associations not only between syntenic loci but also between pairs of unlinked markers on different chromo-

somes. Thus, LD analysis in livestock may require a simultaneous test for linkage and association to avoid false-positive results because of the associations between non-syntenic loci (Farnir *et al.* 2000). Long-range LD was also observed for two bovine genomic regions (chromosomes 4 and 6) in a sample of the dairy cattle population in the UK (Tenesa *et al.* 2003) and for two porcine genomic regions (chromosomes 4 and 7) in five populations of domesticated pigs (Nsengimana *et al.* 2004). Hayes *et al.* (2003) detected LD spanning >10 cM in dairy cattle, and there was extensive LD in the US Holstein population reported by Vallejo *et al.* (2003). These results in livestock species contrast starkly with the extent of LD in human populations, which ranges from 3–5 kb to hundreds of kb (e.g. Pritchard & Przeworski 2001; Ardlie *et al.* 2002; Kaessmann *et al.* 2002). The longer-range LD in livestock than in humans might be caused by evolutionary forces such as genetic drift, admixture, selection and small effective population size, which are common in livestock (Haley 1999).

Although the high levels of LD observed in some livestock species can be exploited for fine-mapping QTL and MAS, little is known about the degree of LD in beef cattle. In Japan, there are two main beef breeds: the Japanese Black and the Japanese Brown. The Japanese Black is famous for high quality meat with prominent intramuscular fat deposition or marbling, whereas the Japanese Brown has a larger mature size and a faster growth rate than the Japanese Black. The objective of this study was to assess and compare genome-wide LD between the Japanese Black and the Japanese Brown cattle breeds.

Materials and methods

Data

The Japanese Black pedigree consisted of one sire and his 1162 half-sib progeny with 31 maternal grand-sires sampled from 30 fattening farms in Oita Prefecture. The progeny were genotyped, but the dams of the progeny were not. A battery of 246 autosomal microsatellite loci was used to measure genome-wide LD. These 246 markers were distributed across 29 chromosomes. The Japanese Brown pedigree consisted of one sire and his 4116 half-sib progeny with 71 maternal grand-sires with known genotypes sampled from 14 fattening farms in Kumamoto Prefecture. The dams of the progeny were not genotyped. A battery of 156 autosomal microsatellite loci dispersed over the genome was used. Microsatellite genotyping was performed as described (Ihara *et al.* 2004). Marker order and interval were determined according to the Shiranakawa USDA linkage map (Ihara *et al.* 2001).

Haplotype reconstruction

Initially, the marker linkage phase of each sire was reconstructed by identifying the frequently cosegregating alleles at linked loci in his half-sib progeny. The paternal allele at each marker was identified in all progeny. In the event that the inherited paternal allele was ambiguous because the sire and his offspring had the same heterozygous genotype, we selected an inherited paternal allele one-by-one conditionally on the transmission probability based on information of the ascertained paternal allele at adjacent marker(s) and the recombination probability between the markers. From this, the paternal haplotype for each offspring was inferred. The maternal allele was inferred by removing the paternal allele from the genotype of offspring. LD was assessed with haplotypes of the dams. Repeating this process 100 times (multiple imputations) resulted in 100 sample sets of maternal haplotypes for every offspring. We assessed LD for the 100 sample sets and obtained estimates by taking the average of 100 values of LD.

LD analysis

Frequencies of alleles and pair-wise haplotypes were estimated from their counts in the maternal gametes within each breed. Both LD coefficients and statistical significance of allelic associations between markers were computed as recommended by McRae *et al.* (2002). The LD coefficient as measured by D' allows simple comparison with the results of Farnir *et al.* (2000).

Following Hedrick (1987), LD between two multiallelic loci A and B was measured as:

$$D = \sum_{i=1}^{r}\sum_{j=1}^{s} p_i q_j D'_{ij},$$

where r and s are the respective number of alleles at the two marker loci, p_i and q_j are the frequencies of marker i allele at locus A and marker allele j at locus B respectively, and D'_{ij} is the absolute value of Lewontin's (1964) normalized LD measure calculated as:

$$D'_{ij} = \frac{D_{ij}}{D_{ij\max}},$$

where $D_{ij} = x_{ij} - p_i q_j$ and

$$D_{ij\max} = \begin{cases} \min[p_i q_j, (1-p_i)(1-q_j)], & D_{ij} < 0 \\ \max[p_i(1-q_j), q_j(1-p_i)], & D_{ij} > 0 \end{cases}$$

with x_{ij} being the frequency of pair wise haplotype $A_i B_j$.

The significance level (α) of allelic associations was estimated using the Monte-Carlo approximation of Fisher's exact test for contingency tables (Slatkin 1994). This approach treats the observed counts of pair-wise haplotypes in a population as a sample of a multinomial distribution and their probability can be obtained from the distribution. The value of α for a given marker pair can be estimated as the cumulative probability of finding a table with the same marginal and total allele counts that has a probability equal to or lower than that of the observed table (Weir 1996). The estimation of α in this study was based on the simulations of 17 000 contingency tables under the null hypothesis of random allelic association.

The computing algorithm was based on the conventional Monte Carlo method (Guo & Thompson 1992) with a minor modification for the test of LD. The observed cumulative frequency distribution of χ-values was compared with that expected under the hypothesis of random allelic association as done by Farnir *et al.* (2000), McRae *et al.* (2002) and Nsengimana *et al.* (2004).

Least-squares analysis was used to test the D' values among syntenic marker pairs, which was used to test for interpopulational and interchromosomal variation in LD. Breed and chromosome were treated as fixed factors and the log-transformed distance between markers was used as a covariate in the general linear model:

$$D'_{ijk} = \mu + b_i + c_j + m_i \log m_k - \log \bar{m} + b_i c_j + b_i m_i \log m_k - \log \bar{m} + c_j m_i \log m_k - \log \bar{m} + \varepsilon_{ijk},$$

where D'_{ijk} is the D' value between two markers separated by distance k on chromosome j in breed i, μ is the average D' value across all the pairs of syntenic loci along the 29 chromosomes in the two breeds, b_i is the effect of breed i, c_j is the effect of chromosome j, m_i is the partial regression coefficient on marker distance, m_k is the genetic map distance k, $\bar{m}$ is the average distance between the markers, and ε_{ijk} is the residual. The value of D'_{ijk} was adjusted by the number of haplotypes according to the model of McRae *et al.* (2002). The $\log_{10}$-transformed distance was used instead of the original distance because of a linear relationship between D' and the log-transformed distance (e.g. McRae *et al.* 2002). Least squares analysis of variance was performed using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC, USA).

Results

The average number of alleles observed in the half-sibs was 6.3 and 6.7 for the Japanese Black and the Japanese Brown respectively, whereas the average heterozygosity of the half-sibs was 0.646 and 0.705 respectively. Total map length and the average interval between markers were 2820 cM (Haldane map) and 12.4 cM for the Japanese Black and 2295 and 20.3 cM for the Japanese Brown respectively.

Linkage disequilibrium was estimated for 11 000 syntenic and 29 135 non-syntenic marker pairs in Japanese Black and 372 syntenic and 13 718 non-syntenic marker pairs in Japanese Brown. Of the 1000 syntenic marker pairs in Japanese Black, 96 pairs were separated by <10 cM and 157 pairs by 10–20 cM. Of 372 syntenic marker pairs in Japanese Brown, 14 pairs were separated by <10 cM and 58 pairs by 10–20 cM.

The extent of LD was measured for syntenic marker pairs within breed. The distribution of the D' value as a function of genetic map distance in the two breeds is shown in Fig. 1a. High levels of LD were found, which ranged over several tens of cM in both breeds. The D' value declined rapidly between 5- and 20-cM spaced markers, gradually between 20- and 40-cM spaced markers, and finally reached a more or less constant value for greater spaced markers. The difference in the D' value between the two breeds was about 0.07 after reaching a constant value. The mean values (SD) of the D' value for syntenic marker pairs were 0.251 (0.109) and 0.163 (0.075) for the Japanese Black and the Japanese Brown, respectively, with a mean difference of

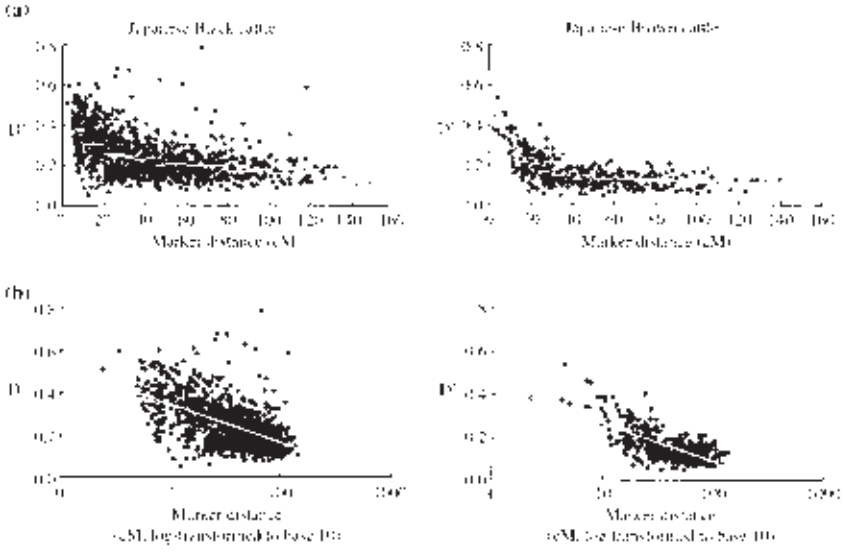

Figure 1 (a) Distributions of observed D' between syntenic marker pairs as a function of genetic map distance (cM) in Japanese Black and Japanese Brown cattle. The grey bars correspond to the average D' values for marker pairs every 5 cM (0–50 cM) or every 10 cM (>50 cM). (b) Distributions of the D' value between syntenic marker pairs as a function of log-transformed marker distance. The grey line is the regression of the D' value on log-transformed marker distance.

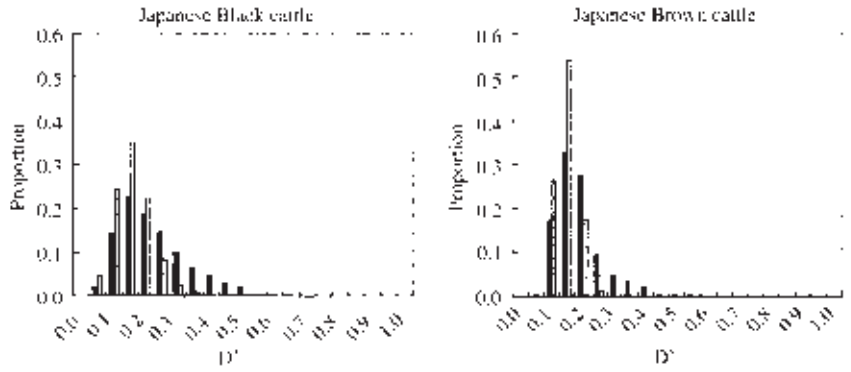

Figure 2 Frequency distributions of the D' value in Japanese Black and Japanese Brown cattle. Syntenic marker pairs are indicated by black bars, while non-syntenic marker pairs are indicated by grey bars.

0.088. The relationship between log-transformed marker distance and the D' value within breed is shown in Fig. 1b. The regression of D' on log-transformed marker distance was $y = -0.1812\ (0.0085)x + 0.527\ (0.0134)$ for the Japanese Black and $y = -0.1785\ (0.0096)x + 0.4444\ (0.0154)$ for the Japanese Brown. The standard error is shown in the parenthesis. Both regression coefficients were highly significant ($P < 0.0001$), suggesting that D' declined significantly as the marker distance increased. There was no significant breed difference in the regression coefficients.

Gametic disequilibrium was evaluated for non-syntenic marker pairs. The mean values (SD) of the D' value for non-syntenic marker pairs were 0.189 (0.080) and 0.122 (0.033) for Japanese Black and Japanese Brown, respectively, with a difference of 0.067 between the two breeds. Although the frequency distributions of the D' value for syntenic and non-syntenic marker pairs overlapped substantially as shown in Fig. 2, the average D' value of syntenic loci was significantly higher ($P < 0.0001$) than that of non-syntenic loci for both breeds according to t test with Welch's correction (Ichihara 2001).

Linkage disequilibrium was tested based on the statistical significance of allelic associations between markers. The cumulative distribution of P-values from the significance tests is shown in Fig. 3. For syntenic marker pairs, P-values were grouped by marker distance. In both breeds, significant LD was observed frequently for marker pairs <40 cM apart, as the cumulative frequency of P-values departed largely from the distribution expected under the random allelic association. Overall, significant LD was observed more frequently in Japanese Brown than in Japanese Black. About 5.5% (about the same frequency as expected by chance) of the studied non-syntenic marker pairs showed significant LD at a 5% significance level in the Japanese Black when compared with 10.8% in the Japanese Brown.

Most of the variation in D' was explained by marker distance ($P < 0.001$; Table 1). The effect of chromosome, the interaction between breed and chromosome, and the interaction between chromosome and marker distance were also significant ($P < 0.001$), while the effect of breed was not significant ($P > 0.05$) (Table 1). Three way interaction of the three main factors was non-significant as reported by Nsengimana et al. (2004) and was excluded from our model.

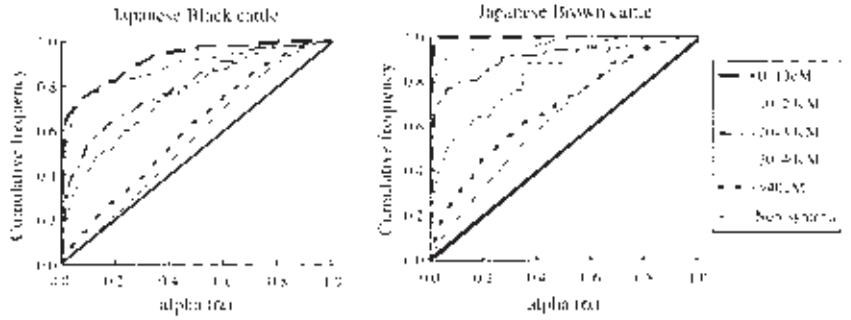

Figure 3 Cumulative frequency distributions of P-values from the significance test of allelic associations between syntenic and between non-syntenic marker pairs in Japanese Black and Japanese Brown cattle. Syntenic marker pairs were grouped by genetic map distance. The diagonal line represents the expected distribution under random allelic association.

Table 1 The least squares analysis table for the *D'* value among syntenic marker pairs

Source of variation	d.f	Mean square
Breed	1	0.000335
Chromosome	28	0.0210*
Marker distance	1	2.122*
Breed × chromosome	28	0.0132*
Breed × marker distance	1	0.00458
Chromosome × marker distance	28	0.0177*
Residual	1284	0.00606

D', linkage disequilibrium coefficient; d.f., degrees of freedom
*$P < 0.001$

Discussion

A genome-wide search for LD was performed on Japanese Black and Japanese Brown cattle. The transmission of maternal haplotypes to the offspring, which were sampled from many paternal half-sib progeny was used to measure LD, where the dams were considered to be a random sample of the female population. Multiple imputations were employed for the haplotype reconstruction of offspring in this study. Comparison of the results between single and multiple imputations indicates that the general pattern of the distribution of the *D'* value between the two methods was similar for each of the two breeds (results not shown). However, the *D'* value between pairs of markers, of which haplotype was ambiguous in more animals, largely fluctuated from imputation to imputation, suggesting that a single imputation must produce biased estimate of the *D'*. In contrast, multiple imputations can account for the uncertainty by means of complementing multiple times to one contingent value (Rubin 1996).

High levels of LD were found among syntenic loci in both breeds. The general pattern of the distribution of *D'* was similar between the two breeds. However, breed difference exists in the mean values of the *D'* value for both syntenic and non syntenic marker pairs and in the *D'* value after reaching a constant value. McRae *et al.* (2002) showed that *D'* can be biased upwards when measured with a small number of haplotypes. These differences correspond approximately with the predicted difference of 0.06 from the model of McRae *et al.* (2002) considering the number of haplotypes in this study. This explained most of the actual difference between two breeds, which may be due to sample size difference. Furthermore, breed difference in average interval between markers could also be a contributing factor in the case of syntenic marker pairs. When the marker distance was lower, the *D'* value was higher (Fig. 1b).

Linkage disequilibrium between non syntenic loci was not significant in Japanese Black, but it was significant in Japanese Brown (about twice as often as expected under random allelic association). Ardlie *et al.* (2002) noted that *P*-values obtained from the test of significant departure from linkage equilibrium between loci depend largely on sample size and even a weak LD could become statistically significant due to a sufficiently large sample. Therefore, the discrepancy in significance tests between the two breeds is also attributable to the difference in sample size rather than in background genotypes.

A genome-wide search by Farnir *et al.* (2000) in dairy cattle showed similar pattern of LD to this study. The observable difference is that LD between non-syntenic loci was highly significant in dairy cattle but not in Japanese beef cattle. The study of Farnir *et al.* (2000) had a greater power of test than this study because they used 581 and 1254 haplotypes when compared with 162 and 406 haplotypes in this study. Nsengimana *et al.* (2004) reported that the difference in statistical significance between their study and Farnir *et al.* (2000) seems to be explained by the number of haplotypes. The results from our study were in agreement with the findings of population-wide LD in dairy cattle (Farnir *et al.* 2000; Hawes *et al.* 2003; Tenesa *et al.* 2003; Vallejo *et al.* 2003) and in other livestock populations (McRae *et al.* 2002; Lou *et al.* 2003; Nsengimana *et al.* 2004) in terms of the level of LD extended over great genetic map distances (about 20–40 cM).

The significant interaction between breed and chromosome indicates that there exists interchromosomal heterogeneity in the LD and that the heterogeneity differs between the two breeds. As described before, the two breeds studied have distinctive, different characteristics in terms of meat productivity and selection history, although they originated from the same native cattle in Japan. The interchromosomal heterogeneity in the LD between the two cattle breeds may result from differential selection, which was in agreement with the report of Nsengimana *et al.* (2004) in pigs.

Historically, the Japanese Black has been subjected to intensive selection for marbling. Using the same half-sib family of the Japanese Black, QTL associated with marbling were detected on seven chromosomes at a 5% chromosome-wise significance level (K. Yokouchi, T. Watanabe, T. Fujita, K. Shiga & Y. Sugimoto, personal communication, 2004). In order to elucidate the relationship between selection and the heterogeneity of the *D'* values, the 29 chromosomes

Table 2 Effect of group of the chromosome with or without region(s) in which QTL for marbling were detected on the *D'* value among syntenic marker pairs

Source of variation	d.f.	Mean square
Group	1	0.0441*
Marker distance	1	3.386**
Group × marker distance	1	0.0298
Residual	996	0.00817

D', linkage disequilibrium coefficient; QTL, quantitative trait loci; d.f., degrees of freedom.
*$P < 0.05$; **$P < 0.001$

were divided into two groups with or without a previously detected QTL region. As shown in Table 2, there was significant difference ($P < 0.05$) between the two groups in the D' value among syntenic marker pairs, indicating that selection for marbling is relating with heterogeneity of the LD between chromosomes in the Japanese Black.

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Radiation hybrid mapping of genes showing intramuscular fat deposition-associated expression changes in bovine *musculus longissimus* muscle

T. Yamada*, Y. Taniguchi*, S. Nishimura*, S. Yoshioka*, A. Takasuga†, Y. Sugimoto† and Y. Sasaki*

*Laboratory of Animal Breeding and Genetics, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
†Shirakawa Institute of Animal Genetics, Odakura, Nishi-shirakawa, Fukushima 961-8061, Japan

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Source/description: Our previous study detected 77 bovine genes (35 annotated genes and 42 novel genes) showing intramuscular fat deposition-associated expression changes in musculus longissimus muscle.[1] In the present study, we mapped nine of the 35 annotated genes and 22 of the 42 novel genes.

Primer sequences and polymerase chain reaction: Polymerase chain reaction (PCR) primers were designed from expressed sequence tag (EST) sequences for all 31 genes (Table S1). PCR amplifications were performed on DNA samples from the 7000-rad Shirakawa Institute-University of Nevada bovine radiation hybrid ($SUNbRH_{7000\ rad}$) panel[2] using standard conditions. PCR products were separated on 3% agarose gels and scored in duplicate.

Chromosomal mapping: PCR amplification of the 31 genes produced the expected bovine-specific fragments. The genes were

Table 1 Assignment of 31 bovine genes using radiation hybrid (RH) mapping, the bovine draft genome sequence and the human genome sequence.

		RH mapping		Bovine draft genome sequence-based mapping			Human genome sequence-based mapping		
EST clone no.	Gene name[1]	Bovine chromosome	Position on the RH map (cR_{7000})	GenBank accession no.[2]	Bovine chromosome	Position (nucleotide)	TC or GenBank accession no.[2]	Human chromosome	Position (nucleotide)
g25-1 no. 2	*BTG2*	16	−13.35	NW_928996	16	2461510	TC289423	1	200006295
g2-21	*DNAJA2*	18	207.51	NW_001016015			TC278331	16	45547900
a20-101	*EDG1*	3	372.88	NW_931090	3	34154494	TC278986	1	101417517
c26-18 no. 2	*MGP*	5	802.6	NW_931408	5	57350935	TC276594	12	14926012
c28-9	*NTAN1*	25	296.73	NW_930388	25	14424841	TC277722	16	15039212
g25-1 no. 1	*PDHB*	22	324.67	NW_930117	22	32065772	TC263319	3	58388043
g22-9	*SPA17*	29	414.23	NW_930734	29	20956144	TC294221	11	124049789
g25-28	*STARD13*	12	214.57	NW_928338			TC292892	13	32576520
c25-22	*TTN*	2	116.71	NW_930833	2	7806329	NT_086633	2	179497655
a6-1	N	28	36.53	NW_930601	28	1875760			
a20-16	N	16	413.58	NW_929050	16	21324800	TC264213	1	12006208
a21-3	N	3	258.63	NW_931053	3	18210723	TC290921	1	142585466
a25-4	N	15	578.79	NW_928969	15	48400094	TC280370	11	46581137
a27-16	N	2	223.93	NW_930837	2	9260020			
c2-11 no. 2	N	10	70.71	NW_972895					
c8-12	N	2	227.98	NW_930876	2	29669660			
c17-25	N	9	646.62	NW_932211	9	34520510			
c19-8 no. 2	N	10	0	NW_976932					
c20-29	N	11	387.49	NW_977487					
c21-8 no. 1	N	14	542.32	NW_928808	14	38709070			
c21-8 no. 2	N	4	604.05	NW_931358	4	51268280			
c22-3	N	20	346.91	NW_988361					
c24-12	N	29	707.13	NW_930782	29	38489990	TC262428	11	64947188
c26-42	N	11	444.18	NW_928316	11	36319690			
c29-46	N	17	566.91	NW_969606					
c30-16	N	X	1166.32	NW_940107					
g5-10	N	7	150.54	NW_931804	7	2957645	TC280323	1	224950814
g8-1	N	4	327.96	NW_942119					
g10-15	N	13	234.9	NW_001000032					
g21-40	N	5	657.8	NW_931554	5	40005957	TC260428	12	100548942
g25-8	N	13	110.96	NW_928567	13	5651840			

[1]N represents novel gene.
[2]The TC or GenBank accession number obtained by searching against TC sequences, human genomic sequences or bovine genomic sequences using the TIGR Cattle Gene Index, the NCBI Human MapViewer or the Bovine MapViewers databases respectively.

typed across the $SUNbRH_{5000\ rad}$ panel and assigned to previously published framework markers[2] at LOD scores >20 using two-point linkage in RHMAPPER (Whitehead Institute/MIT Center for Genome Research, Cambridge, MA, USA). The 'create placement map' option of RHMAPPER was used to place loci on the assigned chromosomes. Table 1 summarizes mapping of the 31 genes on the $SUNbRH_{5000\ rad}$ map.

Comments: Twenty-one of the 31 genes were localized on the bovine draft genomic sequence using the NCBI Bovine MapViewer build 2 version 1 (National Center for Biotechnology Information, Bethesda, MD, USA) (Table 1). The placements of these genes on the $SUNbRH_{5000\ rad}$ map were consistent with their positions in the bovine genome sequence.

Fifteen of the 31 genes were localized to the human genome sequence using the TIGR Cattle Gene Index release 11.0 (The Institute for Genomic Research, Rockville, MD, USA) or the NCBI Human MapViewer build 35 version 1 (Table 1). Placement on the $SUNbRH_{5000\ rad}$ map of 14 of these genes (excluding EST clone g5_101, together with the position obtained by human genome sequence based mapping (Table 1), confirmed conserved segments of synteny on bovine chromosomes 2, 3, 5, 12, 15, 16, 18, 22, 25 and 29.[2]

Mizoshita *et al.*[3] identified a quantitative trait loci (QTL) region affecting intramuscular fat content or 'marbling' on bovine chromosome 4 using a half-sib family of purebred Japanese Black cattle. We mapped EST clone g8–7 within the marbling QTL region on chromosome 4 (Table 1), suggesting that g8–7 may be a candidate for the causal gene involved in marbling in Japanese Black cattle.

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Correspondence: Yoshiyuki Sasaki (sasaki@kais.kyoto-u.ac.jp)

Supplementary Material

The following supplementary material is available for this issue from http://www.blackwell-synergy.com:

Table S1 PCR primer sequences and expected lengths of PCR products for the 31 mapped genes.

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Isolation and characterization of 79 microsatellite markers from the American mink (*Mustela vison*)

R. Anistoroaei*, A. Farid†, B. Benkel†, S. Cirera* and K. Christensen*

*Division of Genetics, Department of Basic Veterinary and Animal Science, The Royal Veterinary and Agricultural University, Grønnegaardsvej 3, 1870 Frederiksberg C, Denmark. †Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, NS, Canada B2N 5E3

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Source/description: Although the American mink (*Mustela vison*) is an economically important species, especially in Northern Europe and in North America, genomic information on this species lags far behind other farmed animals. Microsatellite markers are valuable tools for characterizing the genome, but fewer than 50 microsatellite markers have so far been identified for this species.[1,2] Microsatellites from related species have been used in American mink, such as Eurasian otter (*Lutra lutra*),[3] river otter (*Lutra canadiensis*),[3,4] Eurasian badger (*Meles meles*),[5] marten (*Martes americana*),[2] wolverine (*Gulo gulo*)[1] and American badger (*Taxidea taxus*)[2]. A reference pedigree for mink has not yet been described; therefore, assignment of microsatellites to linkage groups has not been possible.

In this study, additional mink microsatellite sequences were isolated from two genomic libraries constructed by complete digestion with either *Sau*3AI or *Nla*III of approximately 50 μg DNA from one black female mink. Restricted digests were size fractionated on 1% agarose gels, and fragments between 300 and 800 bp and between 800 and 1300 bp were recovered from the gels and purified using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Short and long size-selected fragments from the *Sau*3AI- and *Nla*III-digested DNA samples were ligated into *Bam*HI and *Sph*I digested, dephosphorylated pGEM-7Z vectors (Promega, Madison, WI, USA) and transformed into the XL1-Blue MRF strain of *Escherichia coli* (Stratagene, Cedar Creek, TX, USA).

Insert sequences of 1500 randomly selected clones were obtained using universal primers; they were checked for within- and between-library duplication using BLASTN 2.2.4.[10] Microsatellite repeats were detected using the online 'microsatellite repeat finder' tool available at http://www.biophp.org/minitools/microsatellite_repeats_finder/demo.php. PCR primers were designed using the Primer3 online software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).[11]

Microsatellite characterization: Of the 1500 total sequences, 1343 (90%) were unique and were submitted to the NCBI database (DQ270763–DQ272106). One hundred and thirty-five of the sequenced clones (10%) contained microsatellite repeats; 104 of these microsatellites were chosen for further characterization and were designated starting with *Mvi4000*. Overall, this represents one microsatellite for every 7000 bp. Seventy-nine of the 104 microsatellite sequences contained a convenient microsatellite, 79 were successfully amplified by PCR, and 57 of these (72%) were polymorphic in a panel of 12 unrelated mink of different colour types from two Danish farms (Table 1). An ABI 3100 DNA sequencer equipped with the GeneScan and Genotyper software programs (Applied Biosystems, Foster City, CA, USA) was used for determining the allelic variability of each microsatellite locus.

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Evidence that bovine forebrain embryonic zinc finger-like gene influences immune response associated with mastitis resistance

Mayumi Sugimoto*, **Akira Fujikawa**[†], **James E. Womack**[‡§], **and Yoshikazu Sugimoto**[§¶]

*National Livestock Breeding Center, Nishigo, Fukushima 961-8511, Japan; [†]Hokkaido Animal Research Center, Shintoku, Hokkaido 081-0038, Japan; [‡]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843; and [¶]Shirakawa Institute of Animal Genetics, Nishigo, Fukushima 961-8061, Japan

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Mastitis, a mammary gland inflammation in response to bacterial infection, is a major problem in the dairy industry. We found that cows susceptible to mastitis have a three-base insertion in a glycine-coding stretch of the gene for forebrain embryonic zinc finger-like (FEZL), a transcription factor with a role in neuronal development. Mastitis induces *FEZL* expression in mammary glands, and induced FEZL promotes expression of the axon-attracting molecule semaphorin 5A (SEMA5A) through a GCAG sequence. FEZL also induces *SEMA5A* expression in susceptible cattle but at a lower level than in resistant cattle. Enhanced SEMA5A induces expression of at least nine genes related to the host's immune response, including *TNF-α* and *IL-8*. We propose that susceptibility to mastitis results from an impaired immune response due to the lower transcription activity of susceptible FEZL. Our results provide an avenue to select for genetic improvement of resistance to mastitis and suggest that the FEZL–SEMA5A pathway might control both neuronal development and innate immunity.

FEZL | quantitative trait locus | semaphorin 5A

Mastitis generates annual total losses of ≈69 billion yen in Japan (1) and 2 billion dollars in the United States (2) by reducing milk quantity and quality and increasing health costs. Just as current selective breeding programs based on population genetics have successfully increased milk yield, genetic selection for mastitis resistance could potentially be practiced, not only to reduce the cost of producing milk but also to improve the health and well-being of dairy cows. Recently Wall *et al.* (3) produced transgenic cows resistant to infection by inducing the cells of the mammary gland to secret an antibacterial protein. However, the public may not quickly accept milk from transgenic animals despite the improved health of dairy herds (4). On the other hand, several groups have begun quantitative trait locus (QTL) mapping using a granddaughter design to identify genes influencing mastitis resistance (e.g., ref. 2). Alternatively, we used six half-sibling families whose sires were derived from the same grandsire and performed selective genotyping. This method reduces the number of progeny genotyped, although phenotypic effects may be overestimated because of the biased selection of progeny (e.g., ref. 5). The function of a gene carrying a putative polymorphism positionally identified should significantly influence the phenotype.

Forebrain embryonic zinc finger-like (FEZL) (6) controls the development of monoaminergic neurons (7). Hirata *et al.* (8) reported that FEZL-deficient mice display hyperactive behavior, suggesting that FEZL has a role in neuronal development. Semaphorin 5A (SEMA5A), an axon guidance cue (9), belongs to the semaphorin family characterized by functionally conserved roles in neuronal development (10). Recent studies, however, revealed the existence of crosstalk between neuronal development and immunity. The neuronal repellent Slit regulates both neuronal and leukocyte migration (11). The semaphorin family members SEMA4A and SEMA4D activate T cells (12) and enhance B cell responses (13), respectively. Kumanogoh *et al.* (12, 13) called them "immune semaphorins," but it is not known whether SEMA5A is an immune semaphorin.

We demonstrate here that susceptible cows have a three-base insertion in the *FEZL* gene. Mastitis enhances FEZL, FEZL promotes SEMA5A, and SEMA5A induces *TNF-α* and *IL-8* expression. Because the resistant FEZL allele promotes higher *SEMA5A* expression than the susceptible FEZL allele, the susceptibility to mastitis might result from impaired immune response. Here we identify a gene influencing mastitis resistance at the molecular level and outline an unexpected role for the FEZL–SEMA5A signaling pathway in innate immunity.

Results

A Somatic Cell Score (SCS) Locus Is Linked to FEZL. To determine QTL influencing mastitis resistance, we selected six half-sibling families with a total of 6,561 daughters in the Tokachi county area of Hokkaido, Japan, to minimize possible environmental variations. We used the average SCS [$\log_2$ (somatic cell count/100) + 3] during the first lactation period as a phenotype parameter, because there is a strong genetic correlation between SCS and mastitis (14). The somatic cell count in milk did not normally distribute, whereas SCS did (skewness = 0.93; kurtosis = −0.42; Fig. 5, which is published as supporting information on the PNAS web site). We attempted to collect cows with SCS of ≥5, suspected to have clinical mastitis (15); however, most of them had been culled. The resulting families consisted of 181 daughters with high SCS of 4.82 and 297 daughters with low SCS of 0.65 (Table 1).

With our 478 samples, we embarked on a whole-genome scan of 29 autosomal chromosomes by typing 272 microsatellite markers. The analysis using the DISMULT program of the DISEQ package, which is suitable for a multipoint analysis of selective genotyping data, revealed significant linkage of SCS loci to *Bos taurus* autosomal chromosomes BTA21 and BTA22 with a likelihood of odds (LOD) score of >5 (Fig. 6, which is published as supporting information on the PNAS web site). Further

Conflict of interest statement: No conflicts declared.

Abbreviations: QTL, quantitative trait locus; SCS, somatic cell score; SEMA5A, semaphorin 5A; siRNA, small interfering RNA; TLR, Toll-like receptor; MyD88, myeloid differentiation primary-response protein 88; TRIF, Toll–IL-1-receptor-domain-containing adaptor protein-inducing IFN-β; IRF, IFN-regulatory factor.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AY644769 (resistant) and AY644770 (susceptible) for *B. taurus* forebrain embryonic zinc finger-like (FEZL) protein mRNA; accession nos. DQ335870, DQ335872, DQ335871, and DQ335873 for *B. taurus* fragile histidine triad protein mRNA, *B. taurus* protein tyrosine phosphatase, receptor type G protein mRNA, *B. taurus* hypothetical protein LOC574[illegible] (C3orf14) mRNA, and *B. taurus* synaptoporin protein mRNA, respectively; and accession nos. DQ335869 (resistant) and DQ335868 (susceptible) for *B. taurus* Ca^{2+}-dependent secretion activator protein mRNA].

[§]To whom correspondence may be addressed. E-mail: jwomack@cvm.tamu.edu or kazusugi@siag.or.jp.

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Table 1. SCS of the half-sibling families with the number of collected samples in parentheses

Sire	SCS ≤ 1	1 < SCS < 4	4 ≤ SCS	Total	Average SCS ± SD
1	163 (16)	1,340	751 (7)	2,254	2.67 ± 1.37
2	215 (65)	1,124	601 (30)	1,940	2.56 ± 1.49
3	62 (5*)	418	208 (27)	688	2.55 ± 1.39
4	57 (57)	285	269 (72)	611	2.97 ± 1.60
5	88 (4*)	44*	183 (23)	712	2.41 ± 1.39
6	67 (67)	203	86 (22)	356	2.28 ± 1.48
Total	652 (297)	3,81*	2,098 (181)	6,561	2.60 ± 1.45

analysis with an additional 64 markers in BTA22 supported the linkage (Fig. 1*A*), although the LOD scores by DISLO are likely overestimates because all pedigrees were related. We also confirmed significant linkage as an *F*-statistic of 4.45 ($P < 0.01$) in a total of 6,561 daughters of the families using QTLEXPRESS, which was developed for the analysis of QTL from outbred populations and was able to include the phenotype information of untyped samples (Fig. 1*A*). Next we assumed that the 6.2-centimorgan interval with a LOD score of 3.18 by DISLO is critical for SCS, and we constructed a bacterial artificial chromosome contig containing the region (Fig. 1*B*). Partial sequencing of the contig indicated that the 6.2-centimorgan region was equivalent to *Homo sapiens* autosomal chromosome 3p14.2, where six known genes were assigned (ref. 16 and Fig. 1*B*).

We determined phase of the 6.2-centimorgan region in 478 daughters using four microsatellites and found that Sire 4 had susceptible (S) alleles whereas the remaining sires had resistant (R) alleles, suggesting that Walkway Chief Mark, a grandsire of our families, would have been heterozygous (Fig. 2). Sequencing

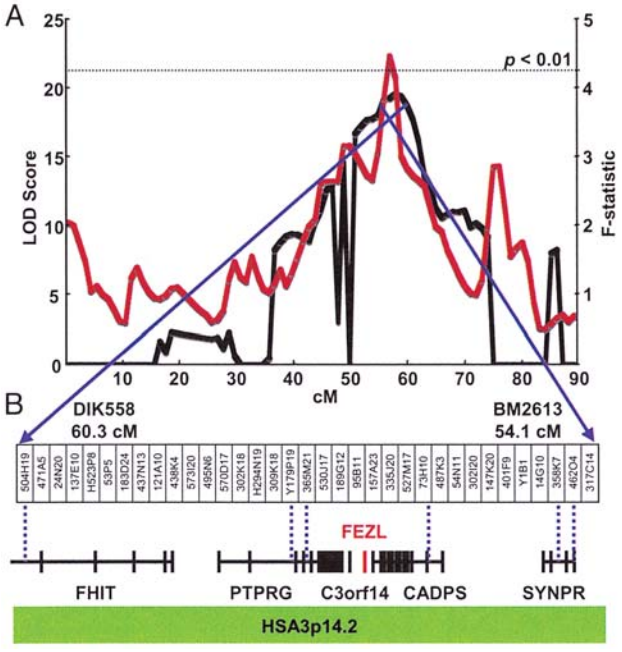

Fig. 1. An SCS locus is linked to FEZL. (*A*) Likelihood of odds score profile by DISLO (black) and *F* statistic profile by QTLEXPRESS (red) on BTA22 obtained by the second screening. A dotted line indicates the chromosome wide threshold of $P < 0.01$ by 10⁴ repeats. (*B*) A bacteria artificial chromosome contig. The names of clones beginning with H were from CHORI-240, names of clones beginning with Y were from Wagyu, and others were from RPCI-42. Blue dotted lines indicate human homologue locations.

exons of the six genes in three cows having homozygous R alleles with low SCS and three cows having homozygous S alleles with high SCS, we identified neither nonsense nor missense mutations except for a three-base insertion in the FEZL S allele. FEZL contains six C2H2 type zinc-finger domains and a glycine stretch where the insertion resulted in an extension of 12 glycine (12G) to 13 glycine (13G) (Fig. 7, which is published as supporting information on the PNAS web site). Although we cannot exclude the possibility that one or more of the other five genes may affect SCS, it is valuable to examine whether the polymorphism in FEZL affects SCS.

In our half-sibling families born during 1981–1996, 8.7% of 12G/12G FEZL cattle had SCS of >5, whereas 17.0% of 13G/13G FEZL cattle did (Table 2), suggesting that 13G/13G FEZL cattle have twice the rate of susceptibility to mastitis than 12G/12G FEZL cattle. Because one sire had 13G/13G FEZL alleles, whereas five had 12G/13G FEZL alleles in our families (Fig. 2), we were able to determine the effect of FEZL genotypes in the six sires on SCS of their offspring. As shown in Table 2, one 12G allele was sufficient to decrease SCS. We also randomly collected and typed 492 samples derived from 217 sires in the Tokachi county area. Although these samples for animals born during 1997–2001 did not include 12G/12G FEZL cattle, the average SCS of 13G/13G FEZL cattle was higher than that of cattle with 12G/13G FEZL (Table 2), supporting the observations in our half-sibling families. Together, these results indicate that 12G FEZL influences resistance to clinical mastitis.

Mastitis Induces FEZL. Consistent with a role for FEZL as a transcription factor that regulates forebrain development, bovine *FEZL* was expressed strongly in the brain and weakly in the mammary gland (Fig. 3*A*). Nevertheless, *FEZL* expression is greater in the quarter affected with mastitis, confirmed by the California Mastitis Test, than in the cured quarter of the same cattle (Fig. 3*A*). Because it is possible that infection enhances *FEZL* expression in the mammary gland, we hypothesized that FEZL has an important role in antimicrobial response and that the polymorphism we described affects function.

FEZL Binds GCAG. To elucidate the effect of the polymorphism on function, V5-tagged FEZL were transfected into monkey kidney COS-7 cells or human breast cancer-derived OCUB-M cells. Both 12G and 13G FEZL localized to the nucleus (Fig. 8, which is published as supporting information on the PNAS web site), indicating that the polymorphism does not affect protein localization.

In many transcription factors, the zinc fingers mediate binding to promoter elements of their target genes (17). To determine the optimal binding sequences for the FEZL protein, we performed chromatin immunoprecipitation assays of cells transfected with His-tagged FEZL. Comparison of the selected sequences by anti-His antibody yielded an optimal binding site of GCAG, and there was no difference between the 12G and 13G FEZL consensus in COS-7 and OCUB-M cells (Fig. 9, which is published as supporting information on the PNAS web site). Gel mobility shift and competition experiments showed that binding was abolished by coincubation with unlabeled consensus (lanes 4 and 9 in Fig. 10, which is published as supporting information on the PNAS web site) but not by AP2 (lanes 5 and 10 in Fig. 10), demonstrating that DNA binding activities of 12G and 13G FEZL are similar.

FEZL Activates SEMA5A. Androgen receptors contain a glycine stretch, and its complete deletion reduces transactivation *in vitro* (18), suggesting that the length of the glycine stretch in FEZL might affect its transcription activity. To compare 12G and 13G FEZL transcription activities, we selected the first (SEMA5A1) and second (SEMA5A2) introns of SEMA5A as binding se-

Marker	INRAMTT178	CSSM41	BMS390	DIK5363	BM2613	DIK612	DIK4524	FEZL	DIK558	DIK2030	BMS875	BMS980	Allele	SCS
cM	47.1	48.9	48.9	52.8	54.1	56	56		60.3	61.9	64.1	64.1		
Walkway Chief Mark	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	2	1	2	4	4	1	2	13G	1	4	4	4	S	
Sire1	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	2	1	1	4	8	2	1	13G	1	3	1	5		
Sire2	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	3	5	2	4	6	4	1	13G	1	4	3	1		
Sire3	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	1	1	1	4	8	2	1	13G	1	3	4	5		
Sire4	2	1	2	4	4	1	2	13G	1	4	4	4	S	
	1	1	2	8	5	1	2	13G	1	3	1	3		
Sire5	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	1	4	1	1	6	2	1	13G	2	3	4	5		
Sire6	2	1	2	4	6	2	1	12G	2	3	4	5	R	
	1	1	2	4	8	2	1	13G	1	2	1	3		
sample 7 from Sire 1	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.9
	2	1	2	4	6	2	1	12G	2	5	2	1	R	
Sequenced sample 1	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.4
from Sire 3	2	1	2	4	6	2	1	12G	2	3	4	5	R	
Sequenced sample 2	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.7
from Sire 3	2	1	2	4	6	2	1	12G	2	3	4	5	R	
Sequenced sample 3	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.4
from Sire 3	2	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 8 from Sire 3	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.9
	2	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 9 from Sire 5	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.9
	2	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 10 from Sire 5	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.7
	1	4	1	1	6	2	1	12G	2	3	4	5	R	
sample 11 from Sire 6	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.9
	1	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 12 from Sire 6	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.9
	2	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 13 from Sire 6	2	1	2	4	6	2	1	12G	2	3	4	5	R	0.6
	2	1	2	4	6	2	1	12G	2	3	4	5	R	
sample 14 from Sire 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	4.4
	1	2	1	4	4	1	2	13G	1	5	2	1	S	
sample 15 from Sire 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	4
	2	1	2	8	4	2	2	13G	1	3	2	3	S	
sample 16 from Sire 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	4.7
	1	4	1	4	4	1	2	13G	1	3	4	2	S	
Sequenced sample 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	4.4
from Sire 4	1	4	1	4	4	1	2	13G	1	4	4	4	S	
Sequenced sample 5	2	1	2	4	4	1	2	13G	1	4	4	4	S	4.1
from Sire 4	1	1	2	4	4	1	2	13G	1	4	4	4	S	
sample 17 from Sire 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	4.5
	1	2	2	4	4	1	2	13G	1	3	1	1	S	
Sequenced sample 6	2	1	2	4	4	1	2	13G	1	4	4	4	S	5.7
from Sire 4	2	1	1	4	4	1	2	13G	1	4	4	3	S	
sample 18 from Sire 4	2	1	2	4	4	1	2	13G	1	4	4	4	S	5.5
	1	2	2	4	4	1	2	13G	1	1	1	1	S	

Fig. 2. Haplotype map of selected samples. Blue and purple boxes indicate homozygous R alleles, and pink and beige boxes indicate homozygous S alleles. Green boxes indicate sequenced samples, and yellow boxes indicate the 6.2-centimorgan region.

quences obtained from the chromatin immunoprecipitation assays (Fig. 11, which is published as supporting information on the PNAS web site). 12G FEZL induces both the SEMA5A1 and SEMA5A2 reporters to higher levels than 13G FEZL (Fig. 3*B*). 12G FEZL stimulates greater *SEMA5A* expression than 13G FEZL in OCUB-M cells (Fig. 3*C*). Conversely, small interfering RNA (siRNA) targeting *FEZL* reduces *SEMA5A* expression (Fig. 12, which is published as supporting information on the

Table 2. The effects of FEZL on SCS

Animals	S ≤ SCS (%)	Average SCS ± SD	*P* value (*t* test)
12G 12G daughters	8 92 (8.7)	1.62 ± 1.84	0.0005*
12G 13G daughters	18 162 (11.1)	2.12 ± 1.98	
13G 13G daughters	38 274 (17.0)	2.56 ± 2.17	0.05**
Daughters from 12G 13G sire	468 5,950 (7.9)	2.56 ± 1.42	
Daughters from 13G 13G sire	73 611 (12.0)	2.97 ± 1.60	0.000001
12G 13G random samples	0 44 (0)	2.00 ± 0.69	
13G 13G random samples	4 448 (0.9)	2.31 ± 0.89	0.01

*, *P* value vs. 13G 13G daughters. **, *P* value vs. 12G, 13G daughters.

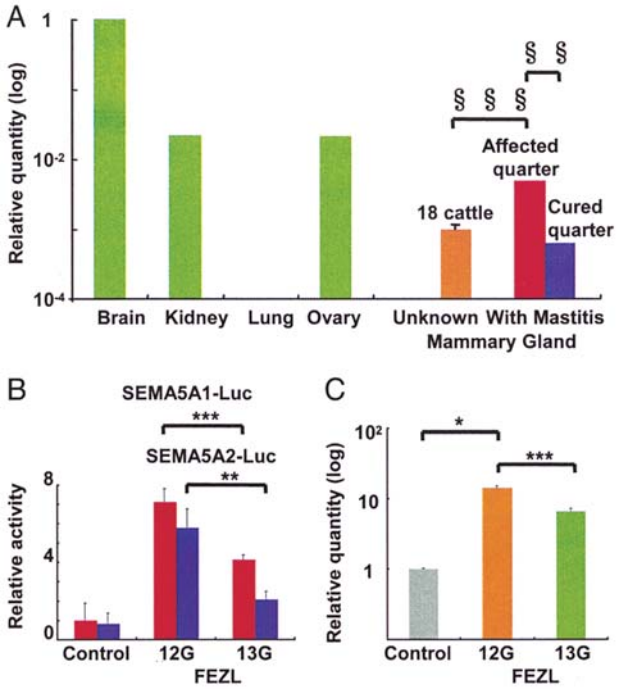

Fig. 3. Mastitis induces SEMA5A through FEZL. (*A*) *FEZL* expression. Results are means of quantity relative to brain. Green bars indicate the mean ($n = 3$) from the same cattle, and red and blue bars indicate the mean ($n = 3$) from other cattle with mastitis. The orange bar indicates the mean ± SEM ($n = 54$) from 18 randomly collected cattle with unknown status. Symbols denote statistically significant results as determined by Student's *t* test: §§, $P < 0.0005$; §§§, $P < 0.0001$. (*B*) Luciferase assay of FEZL with SEMA5A1 (red) and SEMA5A2 (blue). Results are means ± SEM of relative activity to COS7 cells transfected without FEZL. Asterisks denote statistically significant results as determined by Student's *t* test (**, $P < 0.01$; ***, $P < 0.005$). (*C*) *SEMA5A* expression in OCUB-M cells transfected with FEZL. Results are means ± SEM ($n = 9$; three experiments) of quantity relative to OCUB-M cells transfected without FEZL after adjusting for the *FEZL* expression level. Asterisks denote statistically significant results as determined by Student's *t* test (*, $P < 0.05$; ***, $P < 0.005$).

PNAS web site). Moreover, *SEMA5A* expression is greater in 12G/13G FEZL cattle than in 13G/13G FEZL cattle, indicating that SEMA5A is the target of FEZL *in vivo* (Fig. 13, which is published as supporting information on the PNAS web site). Furthermore, mastitis induces *SEMA5A* expression, demonstrating that infection enhances SEMA5A through FEZL *in vivo* (Fig. 13). Thus, the glycine stretch in FEZL appears to influence transcription activity of SEMA5A after infection.

FEZL Induces TNF-α and IL-8 Through SEMA5A. To investigate potential genes that might be induced by SEMA5A, we screened OCUB-M cells transfected with SEMA5A by microarray analysis. The candidate genes induced by SEMA5A included nine genes related to chemotaxis and inflammatory/immune response, but not plexin (PLXN) B3, a gene coding a known receptor for SEMA5A (ref. 19 and Fig. 14, which is published as supporting information on the PNAS web site). The induction of two of the nine genes, *TNF-α* and *IL-8*, was confirmed by quantitative RT-PCR (Fig. 4*A*). In addition, 12G FEZL induced greater *TNF-α* and *IL-8* expression than 13G FEZL (Fig. 4*A*). In contrast, RNA interference of *FEZL* or *SEMA5A* reduced *TNF-α* and *IL-8* expression (Fig. 12). Impaired *TNF-α* and *IL-8* expression controlled by FEZL and SEMA5A could explain susceptibility of 13G FEZL cattle to mastitis.

Infection Induces FEZL Through Toll-IL-1-Receptor-Domain-Containing Adapter Protein-Inducing IFN-β (TRIF). *Escherichia coli* causes 13.9% of mastitis (1). To build a model of mammary glands infected by Gram-negative bacteria, we conducted quantitative RT-PCR using OCUB-M cells after LPS treatment. Consistent with the results in bovine mammary gland (Figs. 5–4 and 13), treatment with 10 μg/ml LPS from *E. coli* induced both *FEZL* and *SEMA5A* expression in OCUB-M cells (Fig. 4*B*). The maximum induction was achieved at 6 h, suggesting that FEZL–SEMA5A signaling is involved in the late phase of the infection. LPS facilitates the myeloid differentiation primary-response protein 88 (MyD88)-dependent response in the early phase and the MyD88-independent response in the late phase through Toll-like receptor (TLR) 4 (ref. 20 and Fig. 4*D*). RNA interference experiments targeting MyD88, TRIF, and IFN-regulatory factor (IRF) 3 revealed that both small interfering TRIF and siIRF3 reduce *FEZL* and *SEMA5A* expression 6 h after LPS treatment whereas siMyD88 does not (Fig. 4*C*). These observations indicated that LPS activates *FEZL* expression in a MyD88-independent manner through the late phase of infection mediated by TLR4, TRIF, and IRF3 (Fig. 4*D*).

Discussion

We have presented multiple lines of evidence demonstrating that *FEZL* is one of the genes responsible for mastitis resistance in cattle. First, the *FEZL* region was mapped as a QTL on BTA22 by using six half-sibling families having the same grandsire. It is important to note that Heyen *et al.* (21) identified seven putative QTL for SCS, including two on chromosome 22. Second, the polymorphism of the length of the glycine stretch of FEZL was significantly associated with SCS. Third, the length of the glycine stretch of FEZL impacts its transcription activity, leading to control of cytokine expression. Our linkage study and cell-based assay clearly indicated that a single glycine insertion into the glycine stretch of FEZL has a large effect on its downstream pathway. In our 1990–1996 samples, the incidence of 13G/13G FEZL homozygotes was 46.9% (224/478). In the 1997–2001 samples, it was 91.1% (448/492). The incidence of clinical mastitis in the Tokachi County area has increased from 19.7–22.5% during 1997–1998 to 24.8–26.2% during 2002–2003 (22), suggesting that changes in FEZL genotypes might have an impact on the incidence of clinical mastitis.

Many studies have documented the importance of neutrophil migration for innate immunity. Neutrophils are highly effective phagocytes for clearing infecting bacterial pathogens from host tissue (23). The number of neutrophils migrating to the site of infection has been associated with the production of IL-8 (24). TNF-α induces *IL-8* expression in the late phase of infection (25), and the IL-8 receptor is also involved in transepithelial neutrophil migration (26). Moreover, lung-specific transgenic mice with KC, one of the IL-8 homologues in the mouse, are resistant to *Klebsiella pneumoniae* infection (27). On the other hand, IL-8 receptor knockout mice are susceptible to acute experimental pyelonephritis (28). Thus, the ability of FEZL to promote *TNF-α* and *IL-8* expression through SEMA5A triggered by LPS represents a plausible mechanism to explain the enhanced antimicrobial activity of FEZL-expressing cells. In addition, we identified FEZL as a QTL influencing mastitis resistance. Our results suggest that FEZL is positioned at the crossroads of neuronal development and innate immunity.

Materials and Methods

Mapping. Genomic DNA was isolated from blood by using NA-1000-48S (Kurabo, Tokyo). Fluorescently labeled (CA)n microsatellite markers were selected from the Shirakawa U.S. Department of Agriculture genetic map (29). Genotyping was performed by using the ABI 3700 sequencer, GENESCAN V3.1.2, and GENOTYPER V2.1 (Applied Biosystems). Linkage analysis

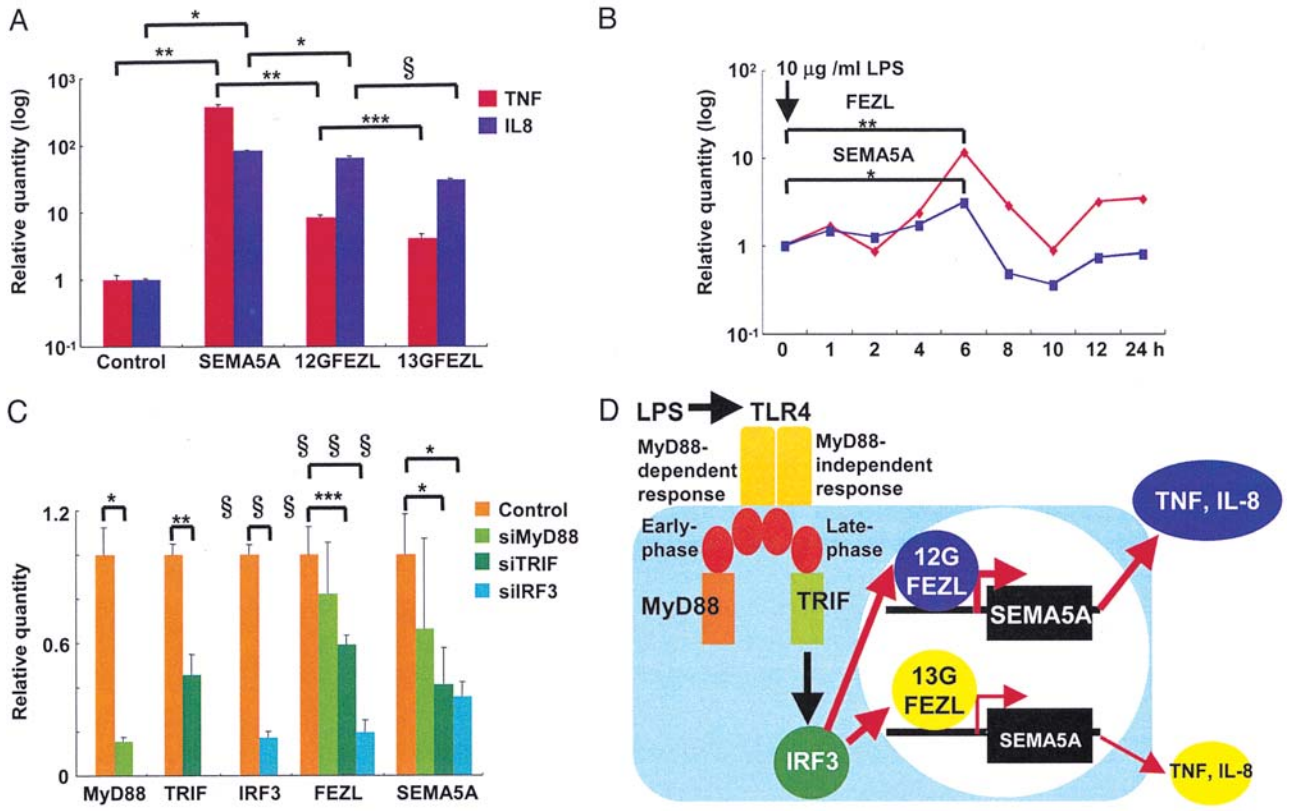

Fig. 4. FEZL's in the TLR4 signaling pathways. (*A*) *TNF* (red) and *IL-8* (blue) expression in OCUB-M cells transfected with SEMA5A or FEZL. Results are means ± SEM ($n = 9$; three experiments) of quantity relative to OCUB-M cells transfected without constructs after adjusting for the *FEZL* expression level. Symbols denote statistically significant results as determined by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; §, $P < 0.0001$). (*B*) *FEZL* (red) and *SEMA5A* (blue) expression in OCUB-M cells treated by 10 μg·ml LPS. Results are means ($n = 3$), and asterisks denote statistically significant results as determined by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$). (*C*) *MyD88*, *TRIF*, *IRF3*, *FEZL*, and *SEMA5A* expression in OCUB-M cells transfected with siRNAs. Results are means ± SEM ($n = 9$; three experiments) of quantity relative to OCUB-M cells transfected without constructs. Symbols denote statistically significant results as determined by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; §§§, $P < 0.0001$). (*D*) Schematic diagram of the TLR4 signaling pathways. Red arrows indicate the pathway revealed by this work.

was done with DISLO (30) and DTEXPRESS (31). To construct a bacterial artificial chromosome contig, we screened the bovine CHORI-240, RPCI-42, and Wagyu bacterial artificial chromosome libraries (32, 33) by PCR. Sequence analysis was conducted by SEQUENCHER V4.1 (Gene Codes, Ann Arbor, MI).

Transfection. Bovine FEZL coding sequences were derived by RT-PCR for exons 1–4 of FEZL by using primers CCTCGGCGGCCACCTCTTCC (forward) and GGAATTCCAGCTCTGCACTGTCCGAGTC (reverse) and by genomic PCR for exon 1, including the glycine stretch of resistant or susceptible FEZL using primers GGGGATCCGGCGCCATGGCAAGCTC (forward) and TGACGCCGGCCGGCTCAGCA (reverse). These coding sequences were connected by BglI (TOYOBO, Osaka) and T4 ligase (New England Biolabs, Beverly, MA). The human SEMA5A coding sequence was derived by RT-PCR using primers AAAAAGCAGGCTCCATGAAGGGAACCTGTGTTATAGCATG (forward) and AGAAAGCTGGGTAGTACTCATCATAATTATTGAGATCTGTAAAGTA (reverse) and human fetal kidney Marathon-ready cDNA (BD Biosciences). Full-length bovine FEZL or human SEMA5A cDNAs were cloned into pcDNA-DEST40 (Invitrogen) to produce V5-His-tagged protein. COS-7 and OCUB-M (34) cells were provided by the RIKEN Bio-Resource Center (Tsukuba, Japan). Transfection was done with Lipofectamine 2000 (Invitrogen) for 24 h for COS-7 and 48 h for OCUB-M cells. Immunofluorescence was performed by using fluorescein isothiocyanate-conjugated V5 antibody (Invitrogen), Vectorshield (Vector Laboratories), Axioplan 2, and Metasystems Isis (Carl Zeiss, Oberkochen, Germany).

Chromatin Immunoprecipitation Assays. Cells transfected with FEZL were processed with the chromatin immunoprecipitation assay kit (Upstate Biotechnology, Lake Placid, NY). Sequences selected by anti-His (C-terminal) antibody (Invitrogen) were amplified in ligation-mediated PCR (35), and the consensus was determined by comparing 20 sequences for 12G FEZL and 16 sequences for 13G FEZL in COS-7 cells and 27 sequences for 12G FEZL and 21 sequences for 13G FEZL in OCUB-M cells.

Gel Mobility Shift Assay. Nuclear protein of COS-7 cells transfected with FEZL was extracted by using the CelLytic NuClear extraction kit (Sigma) and examined by the gel shift assay system (Promega). Protein concentration was measured by Bio-Rad protein assay using BSA as the standard.

Luciferase Assay. Luciferase assay was performed by using a FEZL-pcDNA-DEST40 vector, SEMA5A-pGL3 (R2.2) basic vector, and pRL-TK vector as an internal control (ratio of 10:10:1) based on the dual-luciferase reporter assay system (Promega). Assays were repeated five times each, and the

average measurements were calculated after subtracting the background signals based on 10 readings for *Renilla* luciferase.

Microarray Analysis. RNA of OCUB-M cells transfected with SEMA5A was extracted by using an RNeasy Mini kit (Qiagen, Valencia, CA) and hybridized to Human Genome U133 Plus 2.0 GeneChips according to standard Affymetrix (Santa Clara, CA) protocols. Assays were duplicated.

Real-Time Quantitative RT-PCR. RNA of bovine brain, kidney, lung, mammary gland, ovary, and transfected OCUB-M cells was extracted by using TRIzol (Invitrogen). Quantitative RT-PCR was conducted by using an ABI 7900HT sequence detection system using the comparative Ct method and glyceraldehyde-3-phosphate dehydrogenases (GAPD) as internal controls (Applied Biosystems). RT-PCR with siRNAs was conducted with RNA extracted 6 h after 10 μg/ml LPS treatment. LPS was purchased from Sigma. Primers for RT-PCR are CTACAAGCCCTTCGTCTGTGAAT (forward), GCTGTGGGTCAGCTTGTTGATT (reverse), and ACCAAAAAGGGAACTAC (probe) for bovine FEZL; TGTGGGACCAACGCTTTCA (forward), TCATGGATCTCCGTCAGGTTACT (reverse), and CCTGTCTGCACGAACCGCACGTT (probe) for bovine SEMA5A; and GCCCTCAACGACCACTTTGT (forward), CCTGTTGCTGTAGCCAAATTCA (reverse), and AAGCTCATTTCCTGGTACGA (probe) for bovine GAPD. Sequences of primers for human FEZL, SEMA5A, TNF, IL-8, MyD88, TRIF, IRF3, and GAPD could be purchased as TaqMan Gene Expression Assays (P/N: Hs00375188_m1, Hs00187651_m1, 4327055M, 4327042M, Hs00182082_m1, Hs00706140_s1, Hs00155574_m1, and 4326317E, Applied Biosystems). Sequences for siRNAs for human MyD88 could be purchased as Stealth RNAi (P/N: 46-1564 and 46-1565, Invitrogen). Sequences for siRNAs for human FEZL, SEMA5A, TRIF, and IRF3 could be purchased as siGENOM SMARTpool reagent (P/N: M-016985-00, M-019490-00, M-012833-00, and M-006875-01; Dharmacon, Lafayette, CO).

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Identification of quantitative trait loci affecting corpora lutea and number of teats in a Meishan × Duroc F_2 resource population

S. Sato,*[1,2] K. Atsuji,* N. Saito,‡ M. Okitsu,‡ S. Sato,* A. Komatsuda,† T. Mitsuhashi,*[2] K. Nirasawa,*[3] T. Hayashi,§ Y. Sugimoto,# and E. Kobayashi*

*National Livestock Breeding Center, Nishigo, Fukushima 961-8511, Japan;
†Ibaraki Branch of National Livestock Breeding Center, Fujigaya, Ibaraki 308-0112, Japan;
‡Miyazaki Branch of National Livestock Breeding Center, Kobayashi, Miyazaki 886-0004, Japan;
§National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan; and
#Shirakawa Institute of Animal Genetics, Nishigo, Fukushima 961-8061, Japan

ABSTRACT: Understanding of the genetic control of female reproductive performance in pigs would offer the opportunity to utilize natural variation and improve selective breeding programs through marker-assisted selection. The Chinese Meishan is one of the most prolific pig breeds known, farrowing 3 to 5 more viable piglets per litter than Western breeds. This difference in prolificacy is attributed to the Meishan's superior prenatal survival. Our study utilized a 3-generation resource population, in which the founder grandparental animals were purebred Meishan and Duroc pigs, in a genome scan for QTL. Grandparent, F_1, and F_2 animals were genotyped for 180 microsatellite markers. Reproductive traits, including number of corpora lutea (number of animals = 234), number of fetuses per animal (n = 226), number of teats (n = 801), and total number born (n = 288), were recorded for F_2 females. Genome-wide significance level thresholds of 1, 5, and 10% were calculated using a permutation approach. We identified 9 QTL for 3 traits at a 10% genome-wise significance level. Parametric interval mapping analysis indicated evidence of a 1% genome-wise significant QTL for corpora lutea on SSC 3. Nonparametric interval mapping for number of teats found 4 significant QTL on chromosomes SSC3 ($P < 0.01$), SSC7 ($P < 0.01$), SSC8 ($P < 0.01$), and SSC12 ($P < 0.05$). Partial imprinting of a QTL affecting teat number ($P < 0.10$) was detected on SSC8. Using the likelihood-ratio test for a categorical trait, 2 QTL for pin nipples were detected on SSC2 and SSC16 ($P < 0.01$). Fine mapping of the QTL regions will be required for their application to introgression programs and gene cloning.

Key words: corpora lutea, imprinting, number of teats, pig, quantitative trait loci

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INTRODUCTION

Reproductive performance in sows is regarded as one of the most economically important traits in production of pork. Litter size is a major trait for evaluation of reproductive capability and is affected by component traits, such as ovulation rate, fertilization rate, embryo survival, and uterine capacity (Christenson et al., 1987). The candidate gene approach and QTL analysis have been utilized often to understand the genetic components of such reproductive traits. Rothschild et al. (1996), using a candidate gene approach, were the first to report that the genotype of the estrogen receptor gene was associated with a significant difference in the litter size of pigs. On the other hand, a study that used a whole-genome scan to identify the QTL for ovulation rate in swine (Rathje et al., 1997) detected 4 regions with considerable evidence for QTL segregating on SSC 4, SSC8, SSC13, and SSC15. Rohrer et al. (1999) reported the mapping of QTL for 2 components of litter size (uterine capacity and ovulation rate) using a whole-genome scan; a likely QTL was detected on SSC8, 2 possible QTL for ovulation rate were detected on SSC3 and SSC10, and evidence suggesting the presence of a QTL affecting uterine capacity was found on SSC8.

Our aim was to gain a better understanding of the mechanisms determining litter size by detecting the

[1]Corresponding author: s-shuji@affrc.go.jp
[2]Present address: National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan.
[3]Present address: National Institute of Livestock and Grassland Sciences, Tsukuba, Ibaraki 305-0901, Japan.
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Table 1. Reproductive traits of F_2 animals from an intercross of F_1 derived from a Meishan sow and a Duroc boar

Trait[1]	No.	Avg	SD	Minimum	Maximum
TNB (at 1st parity)	288	9.41	2.71	2	15
CL (at 2nd parity)	234	12.65	3.19	0	21
NF (at 2nd parity)	226	9.72	3.92	0	19
Number of teats	801	15.05	1.20	11	19
Normal = 771, Pn = 30					

[1]TNB = Total number born; CL = number of corpora lutea; NF = number of fetuses per animal; Normal = all nipples functional, and Pn = pin nipples present.

genomic regions affecting some reproductive traits. Here we report the QTL results obtained from analyses of female reproductive traits such as number of corpora lutea (**CL**) and number of teats by using parametric and nonparametric interval mapping methods, assuming Mendelian and imprinted QTL models.

MATERIALS AND METHODS

All the procedures involving animals followed the guidelines for the care and use of laboratory animals established by National Livestock Breeding Center.

Phenotype Measurement

Construction of an F_2 resource population and measurement of phenotypes were performed at the Ibaraki and Miyazaki Branches of the National Livestock Breeding Center. The F_1 generation, consisting of 27 males and 25 females, was produced from crossing a Meishan sow and a Duroc boar, and then a total of 801 F_2 progeny (414 males and 387 females) were produced from 3 F_1 males and 18 F_1 females over 5 farrowings, in which the same F_1 sows were always mated to the same F_1 boars (Sato et al., 2003). Because all F_2 animals were produced in 5 independent groups in different parities and on 2 farms, possible phenotypic differences derived from groups, parities, and farms were removed by preadjusting the phenotypic observations using the appropriate mixed model least squares estimates provided by the maximum-likelihood computer program, as described by Harvey (1977).

The 5 reproductive traits measured are presented in Table 1. Number of teats was measured for 801 F_2 animals at 60 d. Extremely small and nonfunctional nipples were defined as pin nipples (**Pn**). Number of teats was recorded as the sum of the number of functional nipples and Pn. For Pn, each animal was classified into 1 of 2 classes, class 1 and class 2, corresponding to the absence and presence of Pn, respectively. The Pn were thus treated as a categorical trait. Of the 387 F_2 sows, 288 sows (with at least 1 randomly sow selected from each full-sib family) were mated to Large White boars to measure the total number born (**TNB**) at first parity. The F_2 sows were then mated again to Large White boars and were slaughtered at 4 wk of pregnancy. We measured the number of CL and number of fetuses (**NF**) per animal at this second parity. Nonpregnant F_2 sows were removed to measure of CL and NF, resulting in 234 and 226 F_2 sows measured for CL and NF, respectively.

Genotyping

We located 180 informative microsatellite markers at approximately 20-cM intervals in the whole pig genome. These markers were selected from those on the USDA-MARC linkage map (Rohrer et al., 1996). We genotyped the 180 markers for all animals in the resource population (Sato et al., 2003). Extraction of DNA and marker genotyping were carried out as described by Sato et al. (2003).

Linkage Analysis

Linkage maps were constructed by using CRI-MAP (Green et al., 1990) for the 18 autosomes and the method described by Sato et al. (2003) for the sex chromosome. A sex-averaged map was used for the whole-genome scan of QTL. The information content was calculated over the whole-genome region using the method described by Knott et al. (1998).

QTL Analyses

We attempted to detect QTL affecting number of teats, Pn, TNB, CL, and NF. Number of teats, TNB, CL, and NF were discrete traits, and Pn was recorded as a category. The phenotypic values of TNB, CL, and NF were scattered widely and symmetrically, ranging from 2 to 15, from 0 to 21, and from 0 to 19, respectively (Table 1), and were approximately normally distributed. Therefore, these 3 traits were analyzed by a method of interval mapping based on the least-squares method (Haley et al., 1994) by using the following linear model for the phenotypic value of a trait for the ith F_2 individual, y_i, taking imprinting effect into consideration, as described by Knott et al. (1998):

$$y_i = \mu + u_i a + v_i d + w_i f + e_i,$$

where μ is the overall mean, u_i, v_i, and w_i are coefficients for the additive effect, a; dominance effect, d; and imprinting effect, f, respectively, of the QTL at

the tested position, and e_i is the residual error. Denoting the probability of an individual having the genotype AB (i.e., the paternally inherited allele is A and the maternally inherited allele is B) as P(AB), with the genotype of the Duroc boar being QQ and that of the Meishan sows being qq, we can calculate a_i, d_i, and f_i as $a_i = P(\mathrm{QQ}) - P(\mathrm{qq})$, $d_i = P(\mathrm{Qq}) + P(\mathrm{qQ})$, and $f_i = P(\mathrm{Qq}) - P(\mathrm{qQ})$, where it is assumed that the grandparental breeds were fixed for alternative alleles at the QTL. The residual error was assumed to follow a normal distribution with a mean of 0.

Using the least-squares method, we obtained 3 F-ratios, F^1, F^2, and F^3, calculated from the residual sums of squares under the null model, M_0, assuming no QTL; under the full model, M_1, including an imprinting effect in addition to the additive and dominance effects of the QTL; and the Mendelian model, M_2, including only additive and dominance effects without an imprinting effect. We provided F^1, F^2, and F^3 as the F-ratios for 3 comparisons, M_0 vs. M_1, M_0 vs. M_2, and M_2 vs. M_1, respectively. If either or both F^1 and F^2 were significant, a QTL significantly affecting a trait was considered to be detected at the tested position. For the detected QTL, we used F^3 as a test statistic to see whether the QTL was imprinted or not. When the value of F^3 was significant with a genome-wide significance level of 5%, the detected QTL was decided to be imprinted.

For number of teats, the values of which covered only a small range (from 11 to 19, as shown in Table 1), the linear model described above was inappropriate. Therefore, in our analyses of QTL for number of teats we utilized the nonparametric interval mapping (**NPIM**) method, taking imprinting effect into consideration, which is an extension of the method proposed by Kruglyak and Lander (1995), which included only additive and dominance effects. In the NPIM, we considered the correlation between the rank by phenotypic value and the QTL genotype of an F_2 individual. We defined Y_a, Y_d, and Y_f as

$$Y_a = \sum_{i=1}^{n} (n + 1 - 2rank(i))\, a_i,$$

$$Y_d = \sum_{i=1}^{n} (n + 1 - 2rank(i))\, d_i, \text{ and}$$

$$Y_f = \sum_{i=1}^{n} (n + 1 - 2rank(i))\, f_i,$$

where $rank(i)$ denotes the rank by phenotype of the ith F_2 individual. Denoting the variance of a quantity Y by $Var(Y)$, we obtained

$$Z_a = Y_a/\sqrt{\overline{Var}(Y_a)},$$

$$Z_d = Y_d/\sqrt{\overline{Var}(Y_d)}, \text{ and}$$

$$Z_f = Y_f/\sqrt{\overline{Var}(Y_f)}.$$

Under the null model, if $a = d = f = 0$, then Z_a, Z_d, and Z_f are independently normally distributed with a mean of 0 and a variance of 1. If either or both of 2 statistical values, $X_1^2 = Z_a^2 + Z_d^2 + Z_f^2$ and $X_2^2 = Z_a^2 + Z_d^2$, were significant, we determined that a significant QTL was detected. Moreover, we investigated the value of Z_f^2 to see whether the imprinting effect, f, of the detected QTL was significant. The statistics used in the NPIM, X_1^2, X_2^2, and Z_f^2, were regarded as test statistics for comparison of models M_0 vs. M_1, M_0 vs. M_2, and M_2 vs. M_1, respectively.

For the analysis of Pn, which was recorded as a category, an interval mapping method for unordered categorical traits was applied, as described by Hayashi and Awata (2006). In this method, the probabilities of individuals being classified into each category, denoted by P(Class i), where i = 1, 2, were modeled with a logistic regression, where Log[P(Class 2)/P(Class 1)] was expressed as a linear model using the genotype of the QTL affecting Pn as a covariate. The log likelihood-ratio test (**LRT**) statistic was calculated from the maximum likelihood of the null model assuming no QTL and the alternative model assuming the presence of QTL affecting Pn. For the analysis of Pn, the imprinting effect was not incorporated. Moreover, we investigated the segregation ratio of alleles at significant QTL for Pn in Class 2 (the presence of Pn) to determine which alleles derived from Duroc or Meishan caused Pn.

In the analyses of all traits, sex-specific regions on the Y chromosome were assumed to include no QTL and were thus excluded from the analyses. The genome-wide significance thresholds of F-ratios, statistics in NPIM, and LRT were obtained by permutation test (Churchill and Doerge, 1994) of 1,000 repetitions (Table 2). In the detection of QTL, 5% genome-wide thresholds were adopted.

RESULTS AND DISCUSSION

Reproductive Traits Analyzed in Duroc–Meishan Crossbred F_2 Population

The number of animals measured for each analyzed trait is listed in Table 1, along with the means and standard deviations. Measurements were not available for all traits for all animals because of reproductive problems such as infertility.

Results of QTL Analyses

The significant QTL detected are summarized in Table 2. The results of the analyses for each trait are described below.

Total Number Born and Number of Fetuses per Animal. No significant QTL affecting TNB and NF were detected. Rohrer et al. (1999) detected several QTL for female reproductive traits, such as ovulation

Table 2. Summary of QTL locations and additive and dominance effects[1]

	Parametric interval mapping									Nonparametric interval mapping					
	Genome-wise F [2]			Location						Genome-wise χ^2			Location		
Trait	10	5	1	SSC	cM	F-ratio	a	d	% of F_2 variance	10	5	1	SSC	cM	χ^2_1
Mendelian QTL model															
CL	7.67	8.67	10.67	**3**	**36.6**	**12.17****	**-1.27**	**-1.18**	**9.5**						
Number of teats										16.44	16.90	21.72	**3**	**117.0**	**23.49****
													7	**97.1**	**87.51****
													8	**29.7**	**20.76***
													8	**62.9**	**22.60****
													12	**41.4**	**16.94***
Imprinted QTL model															χ^2_2
Number of teats										11.32	12.68	16.51	**8**	**29.7**	**12.86**
Likelihood-ratio test (LRT)															LRT
Pn										6.25	9.82	12.44	**2**	**108.7**	**20.37****
													3	**115.0**	**9.90***
													16	**38.5**	**17.22****

[1]Bold font indicates a significant F-ratio for linkage according to the criteria we used.
[2]Genome-wise F statistic thresholds at the 1, 5, and 10% levels, as determined by permutation test.
[3]Additive (a) and dominance (d) QTL effects correspond to genotype values of $+a$, d, and $-a$, respectively (i.e., individuals that inherited 2 Duroc alleles, individuals with one Duroc and one Meishan allele, and individuals with 2 Meishan alleles). Positive additive effects indicate that Duroc alleles increased the trait, and negative that Duroc alleles decreased it. Dominance effects are relative to the mean of the 2 homozygotes.
*Five percent genome-wise significance level. **One percent genome-wise significance level. CL = number of corpora lutea; and Pn = pin nipples present.

rate and uterine capacity, in a multigeneration Meishan–White composite population. However, they also could not detect QTL for litter size with a threshold set by a nominal significance level of $P = 0.05$. Cassady et al. (2001) reported that the strongest evidence for a number of stillborn QTL was on SSC13 ($P < 0.05$, n = 370). Subsequently, Holl et al. (2004) reanalyzed the same F_2 data and reported that Mendelian QTL on SSC13 ($P < 0.05$), SSC5 ($P < 0.10$), and SSC12 ($P < 0.10$) affecting number of stillborn were identified in single QTL models (n = 370). King et al. (2003) mapped a QTL with a nominal significance level (F-ratio = 4.79) for litter size close to marker *SPP1* on SSC8q in 3 Meishan × Large White cross-populations (n = 152).

Number of Corpora Lutea. We mapped a significant QTL for CL in the interval between *SW72* and *SWR1637* on SSC3 (36.6 cM, $F^1 = 8.11$ and $F^2 = 12.17$; Figure 1). This QTL, however, showed no significant imprinting effect ($F^3 = 0.90$). The estimated additive and dominance effects were $a = -1.27$ (±0.305) and $d = -1.18$ (±0.445), which indicated that the Meishan allele was associated with an increase in CL for the QTL. Rohrer et al. (1999) reported a QTL for ovulation rate on SSC8 and in the same region on SSC3, as a result of genomic scanning in a population derived from a cross between Meishan and a White composite. Ruthje et al. (1997), Wilkie et al. (1999), and Braunschweig et al. (2001) reported that putative ovulation rate or CL QTL were observed on SSC8 at 105, 101, and 99 cM. Cassady et al. (2001) and Holl et al. (2004) observed an ovulation rate QTL on SSC9 at 1 cM. In a previous study, we mapped a significant QTL for testicular weight near *SWR1637* on SSC3 in the same population (Sato et al., 2003). These results together indicate that a region around *SWR1637* is important for reproductive function, i.e., spermatogenesis in the male and follicular maturation in the female.

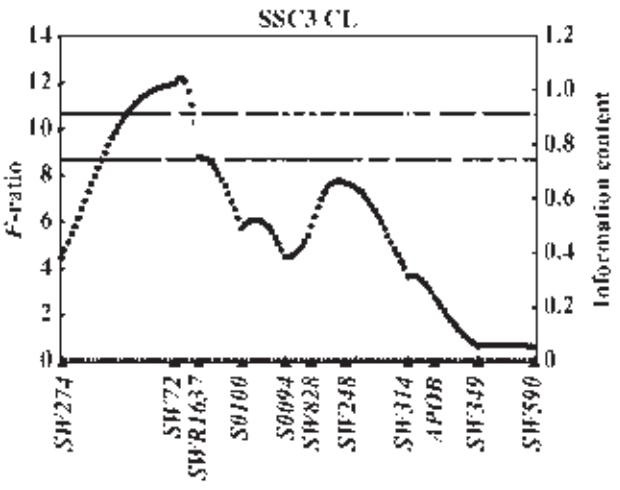

Figure 1. Plot of the F-ratio from multilocus least squares analysis (Haley et al., 1994). The x-axis indicates the relative position in the linkage map. The left y-axis and right y-axis represent the F-ratio from the Mendelian model and information content, respectively. A triangle on the x axis indicates a marker position. Horizontal lines indicate threshold values for genome-wise 5% level (thin line) and genome-wise 1% level (thick line). The curved line indicates information content, and ● = CL.

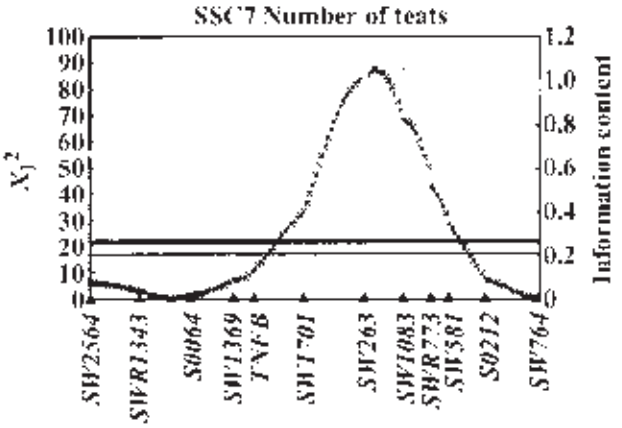

Figure 2. Plot of the X_1^2 from nonparametric QTL mapping with Mendelian model (Kruglyak and Lander, 1995). The x-axis indicates the relative position in the linkage map. The left y-axis and right y-axis represent the χ^2 statistic (X_1^2) from the Mendelian model and information content, respectively. A triangle on the x-axis indicates a marker position. Horizontal lines indicate threshold values for genome-wise 5% level (thin line) and genome-wise 1% level (thick line). The curved line indicates information content, and + = number of teats.

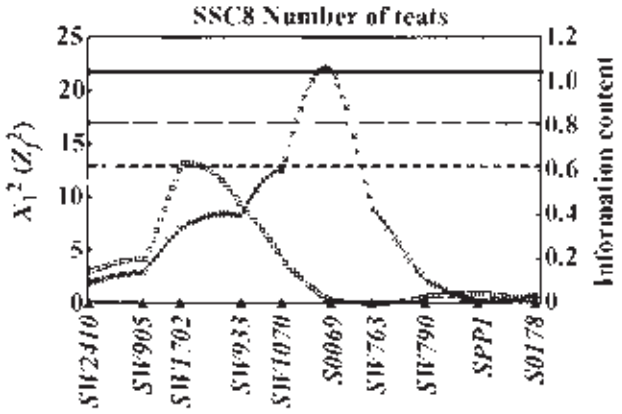

Figure 3. Plot of the X_1^2 and Z_1^2 from nonparametric QTL mapping with the Mendelian and imprinting models, respectively (Kruglyak and Lander, 1995). The x-axis indicates the relative position in the linkage map. The left y-axis and right y-axis represent the χ^2 statistics (X_1^2 and Z_1^2) and information content, respectively. A triangle on the x-axis indicates a marker position. Horizontal lines indicate threshold values for genome-wise 5% level (thin line), genome-wise 1% level (thick line). The dash line indicates threshold values for genome-wise 5% level with the imprinting model. The curved line indicates information content, + – number of teats with the Mendelian model, and – number of teats with the imprinting model.

Teat Number and Pin Nipples. Counting of the nipples revealed that F_2 animals could be classified into pigs with all functional nipples (n = 771 animals; class 1) and those with some Pn (n = 30 animals; class 2). The number of teats was scored as the sum of the numbers of functional nipples and Pn. We analyzed the QTL affecting teat number by the NPIM method and those affecting Pn by an interval-mapping method suitable for unordered categorical traits. We detected 5 QTL affecting number of teats in the regions between *APOB* and *SW349* on SSC3 (X_1^2 = 23.49 at 117.0 cM), between *SW263* and *SW1083* on SSC7 (X_1^2 = 87.51 at 97.1 cM; Figure 2), between *SW1702* and *SW933* on SSC8 (X_1^2 = 20.76 at 29.7 cM), between *SW1070* and *S0069* on SSC8 (X_1^2 = 22.60 at 62.9 cM; Figure 3), and between *SW1307* and *SW874* on SSC12 (X_1^2 = 16.94 at 41.4 cM). Of these 5, only the QTL located at 29.7 cM on SSC8 QTL showed a significant imprinting effect (Z_1^2 = 12.86). Although it is generally difficult to resolve 2 linked QTL on a chromosome by the single QTL model adopted here, the 2 QTL detected on SSC8 showed different gene action, one with imprinting and the other without imprinting; we therefore considered that we had successfully detected them separately. In NPIM, the direction of the effect of the allele from each grandparent can be estimated by the signs of Z_a and Z_d. The Duroc allele had the effect of increasing number of teats at the QTL on SSC7 (Z_a = 9.35), whereas the Meishan alleles had the effect of increasing the number of teats at 3 other QTL (Z_a = –3.15, Z_a = –4.30, and Z_a = 3.51 for the QTL on SSC3, at the one at 62.9 cM on SSC8, and the one on SSC12, respectively). We previously mapped a QTL affecting vertebral number at 98.1 cM on SSC7 and found that Duroc alleles had the effect of increasing the number of vertebrae (Sato et al., 2003). It is likely that a gene responsible for vertebral number can be utilized to improve the number of teats.

For the QTL with a significant imprinting effect on SSC8, we obtained Z_d = 3.51, which indicated that the paternally inherited allele of Duroc origin was expressed more strongly than the maternally inherited one; or, conversely, that the maternally inherited allele of Meishan origin was more strongly expressed than the paternally inherited one. Searches for QTL affecting number of teats have been reported, but none of the results of these studies agreed with ours.

Using LRT, we detected 3 QTL affecting Pn on SSC2, 3, and 16. On the markers adjacent to the highly significant QTL ($P < 0.01$), *SW1879* and *SW2192* on SSC2 and *SW403* and *SWR2086* on SSC16, the χ^2 test indicated that segregation at QTL in all F_2 animals followed the expected ratio (1:2:1) but that the segregation in Pn animals was significantly different from the expected ratio with the increased frequencies of Duroc

Table 3. Allele frequency on Pn QTL in chromosomal regions exceeding a 1% genome-wise significance level[1]

	SSC2				SSC16			
Alleles	*SW1879* (102.1 cM)		*SW2192* (116.2 cM)		*SW403* (27.6 cM)		*SW2086* (38.7 cM)	
	F_2	Pn	F_2	Pn	F_2	Pn	F_2	Pn
Duroc homozygous	205	14	210	18	206	13	205	16
Heterozygous	413	7	382	6	394	10	394	7
Meishan homozygous	181	9	197	6	201	7	201	7
Total	799	30	799	30	801	30	800	30
X^2	2.35	10.20	0.70	20.40	0.27	5.73	0.22	13.93
P	0.3–0.5	0.001–0.01	0.5–0.7	<0.001	0.8–0.9	<0.001	0.8–0.9	<0.001

[1]F_2 = All F_2 animals; and Pn = pin nipples present.

alleles (Table 3). This result might indicate that a Duroc founder had a causative gene for Pn, although it is difficult to come to a clear conclusion because of small number of Pn animals (30 in 801 animals). Identification of the causative gene for Pn could be of practical use in pig breeding programs for excluding genetic diseases, as has been done in the case of mutations in the *ryanodine receptor 1* (*RYR1*) gene (Fujii et al., 1991).

In this study, we detected a QTL for CL, 5 QTL for number of teats, and 3 QTL for Pn – only one of which showed a significant imprinting effect. Generally, the heritability of reproductive traits such as litter size is low, so the use of larger resource families is probably necessary if we are to increase our power to detect QTL. Several QTL studies that targeted reproductive traits in swine, such as that of Rohrer et al. (1999), identified QTL that agreed with ours. Even if a gene responsible for CL was identified and used for selection, successful improvement in litter size would not necessarily be attained. Litter size is entwined with multiple factors such as environmental influences, ovulation rate, fertilization rate, embryo survival, and uterine space. However, the number of corpora lutea is not irrelevant to litter size, and it is quite likely that increasing CL would be one of the most effective ways of increasing litter size. In addition to classical breeding, identification of the responsible genes underlying the QTL by candidate gene approaches might be successful in improving reproductive traits by the use of DNA-level information.

IMPLICATIONS

Our findings provide basic information on the transmission of some porcine quantitative traits. Clarification of the genes responsible for reproductive traits requires the use of algorithms to analyze gene interactions because these traits are multifactorial. Several of the quantitative trait loci identified in this study could be promising targets for marker-assisted selection.

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FULL PAPER *Internal Medicine*

Carrier Rate of Factor XI Deficiency in Stunted Japanese Black Cattle

Daisaku WATANABE[1], Takashi HIRANO[2], Yoshikazu SUGIMOTO[2], Yoshimi OGATA[3], Shogo ABE[3], Takaaki ANDO[1], Hiromichi OHTSUKA[1], Tetsuo KUNIEDA[4] and Seiichi KAWAMURA[1]

[1]*School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034–8628,* [2]*Shirakawa Institute of Animal Genetics, Odakura, Nishigo, Nishi-Shirakawa, Fukushima, 961–8061,* [3]*NOSAI Yamagata Veterinary Training Center, 286–1 Kitagawara, Yamagata 990–2171,* [4]*Graduate School of Natural Science and Technology, Okayama University, Tsushima-naka, Okayama 770–8530, Japan*

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ABSTRACT: Blood examinations and genotyping of Factor XI (*F11*) were performed in growth retardation Japanese Black cattle and their dams. Genotyping of *F11* revealed that the recessive homozygous and heterozygous genotype frequencies were 5.2% and 50.0% in the *Claudin 16* (*CL-16*) deficiency group (n=58), 0% and 14.2% in the renal dysplasia group (n=7), 0% and 26.1% in the non *CL-16* deficiency nephritis group (n=23), 8.9% and 46.7% in the hypogenesis syndrome group (n=45), 6.2% and 25.0% in the neonatal weak calf syndrome group (n=32), 9.2% and 38.6% in the respective dams group (n=44), 0% and 23.1% in the normal cattle group (n=13), and 5.9% and 38.2% in total (n=222), respectively. These results showed that the carrier rate of *F11* deficiency was high in Japanese Black cattle, and that the *CL-16* deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups had a large amount of recessive homozygous genotype than the other groups. No abnormal bleeding was observed clinically in the present study, and 4 of the recessive homozygous dams showed normal growth and parturition.

KEY WORDS: *claudin 16* deficiency, factor XI deficiency, growth retardation, hypogenesis, Japanese Black cattle.

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Calves with abnormal development caused by *Claudin 16* (*CL-16*) deficiency, which is known to cause hereditary renal failure [7, 12–15, 18, 19, 28, 29], non-*CL-16* deficiency nephritis, which genotyping is not *CL-16* deficiency in spite of similarity to *CL-16* deficiency in its clinical symptoms, hematological aspects, and pathological findings [21, 22], renal dysplasia, which is suspected to be a hereditary disease [23], neonatal weak calf syndrome [15], hypogenesis syndrome, and other defects are frequently seen in Japanese Black cattle. It has been suggested that neonatal weak calf syndrome is associated with asiderotic anemia and immunodeficiency [16], and that hypogenesis syndrome is associated with growth hormone (GH) deficiency [17], insulin like growth factor-I (IGF-I) deficiency in contrast with hypersecretion of GH [5, 6, 11, 26], primary or secondary underactive thyroid function [5, 6, 24, 30] and other factors.

Factor XI (*F11*) is a factor of intrinsic blood coagulation that is produced in the liver. *Factor XI* deficiency is inherited in an autosomal recessive or dominant manner [1], and it causes a congenital bleeding disorder characterized by minor bleeding episodes and severe protracted bleeding after trauma or surgical procedures in humans [20].

The first case of *F11* deficiency in Holstein Friesian cattle was reported by Kociba *et al.* [8], and in that case, the inheritance of *F11* activity in the Holstein Friesian cattle was autosomal dominant, with severe deficiency in homozygotes and partial deficiency in heterozygotes [2]. Recently, a causative mutation for *F11* deficiency in Japanese Black cattle was identified by Kunieda *et al.* [9], making genotype analysis of the *F11* gene possible. *Factor XI* deficiency with mild bleeding and severe deficiency of *F11* activity were detected in Japanese Black cattle [9, 25].

The authors studied the hematological aspects and genotype frequencies of *F11* deficiency in abnormally developed cattle, dams, and normal cattle groups, and attempted to unravel the associations between retarded growth and *F11* deficiency in Japanese Black cattle.

MATERIALS AND METHODS

Animals: Cattle with abnormal growth (n=165) were divided into 5 groups for this study based on clinical symptoms, hematological characteristics, pathological findings, and genotyping of *CL-16* deficiency as follows: *CL-16* deficiency (n=58), renal dysplasia (n=7), non-*CL-16* deficiency nephritis (n=23), hypogenesis syndrome (n=51), and neonatal weak calf syndrome (n=26). In addition, 2 groups, dams of stunted cattle (n=44) and normal cattle (n=13), were examined for hematological aspects and the genotype of *F11* deficiency (Table 1).

CL-16 deficiency was examined using the DNA-based tests developed by Hirano *et al.* [4]. Renal dysplasia was diagnosed from pathological findings. Non-*CL-16* deficiency nephritis was used as the category for cases that were not *CL-16* deficiency according to the DNA based tests despite the similarity to *CL-16* deficiency in clinical symptoms, hematological characteristics, and pathological findings. Hypogenesis syndrome was used as the category for cases of normal appearance without congenital atelosis. The cattle used were determined to be free of BVD-MD infections and did not show symptoms of the aftereffects of diseases such as pneumonia and diarrhea. A number of the cattle with hypogenesis syndrome cattle had ongoing neona-

Table 1. Characterization of the stunted cattle groups, their dams and normal cattle

Cattle group	Female heads	Male heads	Total heads	Mean month age (ranges)	Mean BWI[a] (ranges)	Main symptoms
Claudin-16 deficiency	34	24	58	13.8 (1–38)	72.7 (31–93)	Anorexia, depression, long hooves (about 65%), chronic interstitial nephritis
Renal dysplasia	4	3	7	10.9 (0–31)	38.7 (21–53)	Ateliosis, depression, inability to nurse, renal dysplasia, shorting of lower jaw (about 60%)
Non-*Claudin-16* deficiency nephritis	12	11	23	13.8 (1–27)	66.3 (37–87)	Anorexia, depression, long hooves (about 50%), chronic interstitial nephritis
Hypogenesis syndrome	26	19	45	11.6 (3–31)	56.6 (26–79)	No sign without lower growth
Neonatal weak calf syndrome	16	16	32	1.5 (0–3)	48.8 (17–82)	Weakness, depression, inability to nurse or anorexia, diarrhea (partly)
Dams of stunted cattle	44	0	44	63.8 (24–218)	98.2 (88–114)	No sign
Normal cattle	9	4	13	17.2 (1–29)	97.6 (86–120)	No sign
Total	145	77	222			

a) Body Weight Index: Percentage to the standard body weight of Japanese Black cattle in the same month

tal weak calf syndrome. Neonatal weak calf syndrome was used as the category for cases of weakness of unknown cause in the neonatal period, and in some animals in this category, anorexia, depression, staggering, diarrhea, and anemia were observed. However, several cases of neonatal weak calf syndrome have been found with ongoing weakness until 2–3 months of age. Therefore, this syndrome was diagnosed during the nursing period in this study.

Clinical screening: We investigated the form of manifestation of the defects and performed familial screening for these affected cattle. Body weight index (BWI) was expressed as a percentage of the standard body weight of Japanese Black cattle [27] in the same month.

Blood samples were collected from the jugular vein and mixed with a small volume of heparin sodium for laboratory tests, complete blood work using an automatic blood cell counter, and biochemistry tests using an autoanalyzer (Hitachi 7170S, Tokyo) for total protein (TP), albumin (Alb), total cholesterol (T. chol), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), urea nitrogen (UN), creatinine (Cre), calcium (Ca), inorganic phosphorus (iP), and magnesium (Mg).

Genotype analysis: Heparinized blood samples stored at −40°C were used for genotyping. Genotype analyses for *FH* deficiency were performed using the DNA-based tests developed by Kunieda *et al.* [9].

Statistics: The differences between each group and the normal cattle in the blood examination were compared using the Student's *t* test for the homoscedastic items, and using the *Cochran-Cox* methods for items with different variances. A p-value of less than 0.05 was considered to indicate a significant difference.

RESULTS

Clinical screening: The cattle and findings of clinical screening used in the present study are listed in Table 1. Clinical symptoms included insufficient growth, anorexia, depression, and long hooves in the majority of cattle with chronic interstitial nephritis in the *CL-16* deficiency and non-*CL-16* deficiency nephritis groups. The mean body weight index (BWI) for both groups was 66–73%, which was higher than that in the other stunted groups (39–66%), and several cattle in the *CL-16* deficiency group had almost normal BWI. Most of the cattle in the *CL-16* deficiency and non *CL-16* deficiency nephritis groups first showed failure to thrive at 5 months old or later, and thus the age at which this abnormality was identified was later than in the other stunted groups. All calves in the renal dysplasia group had marked ateliosis (mean BWI: 39%), depression, and inability to nurse with congenital marked dysplasia of the kidneys (0–60% of the weight of normal kidneys), and most of these calves also had shortening of the lower jaw. The cattle in the hypogenesis syndrome group had no anamnesis, but did show insufficient growth (mean BWI: 57%). In the neonatal weak calf syndrome group, clinical symptoms included weakness, depression, inability to nurse or anorexia and depression in all cattle, and insufficient growth (mean BWI: 49%) and diarrhea were observed on occasion.

In the present study, no cattle with abnormal bleeding were clinically observed in spite of the fact that 13 cattle had *FH* deficiency, and their dams displayed normal growth, reproduction, and parturition. The cattle in the *CL-16* deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups were within the same family as the cattle in the *FH* deficiency group.

Table 2. Hematological aspects of the stunted cattle groups, their dams and normal cattle

Cattle group		RBC $10^4/\mu l$	WBC $10^2/\mu l$	Ht %	TP g/dl	Alb g/dl	T.chol mg/dl	AST IU/l	GGT IU/l	UN mg/dl	Cre mg/dl	Ca mg/dl	iP mg/dl	Mg mg/dl
CL-16 deficiency (n=58)	Mean	721.7**	87.6	32.7*	6.9	3.6	174.9**	210.0*	32.0	90.3**	6.3**	8.1*	12.2**	2.9
	SE[a]	31.1	5.3	1.1	0.1	0.1	10.6	46.6	7.6	5.7	0.6	0.3	0.6	0.2
Renal dysplasia (n=7)	Mean	875.0	87.3	36.8	6.4	2.9*	97.7	145.7	77.5	82.3**	7.3**	8.6	12.9**	2.7
	SE	72.3	19.9	4.1	0.6	0.1	37.9	77.0	32.2	19.6	0.8	1.8	1.8	0.2
Non-*CL-16* deficiency nephritis (n=23)	Mean	783.8	97.5	32.8	7.7	3.2	85.9	190.2	33.7	99.0**	8.6**	8.3**	11.9**	3.9**
	SE	67.9	11.2	2.7	0.4	0.2	13.0	41.1	6.4	12.5	1.6	0.5	1.1	0.4
Hypogenesis syndrome (n=45)	Mean	812.5	85.5	35.2	6.6	3.0**	96.6	105.8	31.8	13.8	1.0	9.9	7.7	2.3
	SE	29.8	6.3	1.2	0.2	0.1	3.1	18.8	4.1	0.8	0.1	0.1	0.2	0.1
Neonatal weak calf syndrome (n=32)	Mean	711.2*	82.4	28.2*	5.5*	2.9**	86.0	151.5	91.0	17.5	1.1	10.8	8.6	2.2
	SE	66.5	9.0	2.0	0.2	0.2	10.9	36.1	60.5	2.0	0.1	0.2	0.3	0.1
Dams of stunted cattle (n=44)	Mean	835.4	80.7	39.1	7.5	3.5	103.5	75.7	19.0	11.9	1.1	9.1	5.1**	2.4
	SE	67.5	8.3	2.2	0.2	0.2	14.3	9.2	3.0	2.7	0.1	0.3	0.5	0.2
Normal cattle (n=13)	Mean	912.9	90.0	36.3	6.5	3.5	96.9	91.2	19.4	14.9	1.0	10.0	8.8	2.3
	SE	60.5	6.2	2.5	0.2	0.2	12.0	12.5	3.8	1.4	0.1	0.3	0.5	0.2

a) SE: Standard error.
The asterisk indicates a significant difference from the value in normal cattle: *p<0.05, **p<0.01.

Table 3. The genotype frequencies of factor XI deficiency (*F11D*) in the stunted cattle groups, their dams and normal cattle

Cattle group	Heads	*F11*D normal homo type (%)	*F11*D hetero type (%)	*F11*D recessive homo type (%)
Claudin-16 deficiency	58	26 (44.8%)	29 (50.0%)	3 (5.2%)
Renal dysplasia	7	6 (85.8%)	1 (14.2%)	0 (0%)
Non-*Claudin-16* deficiency nephritis	23	17 (73.9%)	6 (26.1%)	0 (0%)
Hypogenesis syndrome	45	20 (44.5%)	21 (46.7%)	4 (8.9%)
Neonatal weak calf syndrome	32	22 (68.8%)	8 (25.0%)	2 (6.2%)
Dams of stunted cattle	44	23 (52.3%)	17 (38.6%)	4 (9.1%)
Normal cattle	13	10 (76.9%)	3 (23.1%)	0 (0%)
Total	222	124 (55.9%)	85 (38.2%)	13 (5.9%)

In the blood examinations, the number of red blood cells (RBC) and hematocrit (Ht) values were lower in the *CL-16* deficiency and neonatal weak calf syndrome groups (Table 2). Plasma UN, Cre, and iP were significantly higher, and plasma Ca was significantly lower in the *CL-16* deficiency, renal dysplasia, and non-*CL-16* nephritis groups compared with the normal cattle. Plasma T. chol was significantly higher in the *CL-16* deficiency group. Plasma Alb was significantly lower in the hypogenesis syndrome group, and TP and Alb were significantly lower in the neonatal weak calf syndrome group.

Genotype analysis: As shown in Table 3, genotyping of *F11* revealed that the recessive homozygous and heterozygous genotype frequencies were 5.2% and 50.0% in the *CL-16* deficiency group (n=58), 0% and 14.2% in the renal dysplasia group (n=7), 0% and 26.1% in the non-*CL-16* deficiency nephritis group (n=23), 8.9% and 46.7% in the hypogenesis syndrome group (n=45), and 6.2% and 25.0% in the neonatal weak calf syndrome group (n=32), respectively. Furthermore, the recessive homozygous and heterozygous genotype frequencies were 9.1% and 38.6% in the dams of stunted cattle group (n=44), 0% and 23.1% in the normal cattle (n=13), and 5.9% and 38.2% in the total cattle (n=222), respectively.

These results show that the rate of carriers for *F11* deficiency was high in Japanese Black cattle, and that the *CL-16* deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and their dams groups had higher rates of recessive

homozygous genotype than the other groups.

DISCUSSION

In this study, it was demonstrated that the rate of carriers for *F11* deficiency is high in Japanese Black cattle. The reason for this high rate of carriers for *F11* deficiency in the *CL-16* deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups was thought to be that the families of these groups were within the same family as the *F11* deficiency. This family of affected cattle is known for its excellent beef marbling score and meat grade, and for that reason, the semen of the sires in this family is widely used for artificial insemination in Japan. In fact, the affected cattle were inbred in this family, and consequently, it is suggested that these diseases occur often in the population of Japanese Black cattle.

In humans, *F11* deficiency is inherited in an autosomal recessive or dominant manner [1]. In cattle, a mutation that prevents homodimer formation usually results in recessive inheritance, since significant amounts of normal homodimer can be formed by the products of the wild-type alleles in the heterozygous genotype, and mutations in the catalytic domain of proteins usually result in dominant inheritance by formation of heterodimers of the mutant and wild type polypeptides [9].

Kunieda *et al.* [9] reported that Japanese Black cattle with *F11* deficiency showed a marked reduction in *F11* activity (less than 10% of the normal activity) with prolonged bleeding time and abnormal plasma coagulation. Takasu *et al.* [25] also reported an *F11* deficiency case in a 1 month old Japanese Black calf showing growth retardation, hip dysplasia and abnormal bleeding with prolongation of activated partial thromboplastin time (APTT) and low *F11* activity.

In the present study, 13 heads of cattle, including 4 dams, were identified as having the *F11* deficiency recessive homozygous genotype; however, none of them had shown abnormal bleeding in the past. Therefore, it is possible that the cattle with *F11* deficiency normally show only mildly abnormal bleeding.

On the other hand, Canadian Holstein cattle with *F11* deficiency showed a lower rate of fetal and calf survival, higher susceptibility to infectious disease, and higher frequency of being repeat breeders [10]. Furthermore, one Holstein cow with repeat breeding has been found to have the *F11* deficiency heterozygous genotype in Japan [3]. However, no dams showed repeat breeding or abnormal bleeding at parturition in the *F11* deficiency dams in the present study, and the rate of carriers was very high in the Japanese Black cattle. Therefore, *F11* deficiency might have little effect on the reproductive ability of Japanese Black cattle. However, further investigations will be necessary to determine the differences of symptoms between Japanese Black and Holstein *F11* deficiency cattle.

The percentages of cattle with the normal homozygous *F11* deficiency genotype in the hypogenesis syndrome and neonatal weak calf syndrome groups were 45% and 69%, respectively. For this reason, it seems that *F11* deficiency is not directly associated with these syndromes.

The cattle with *F11* deficiency in combination with *CL-16* deficiency, hypogenesis syndrome and/or neonatal weak calf syndrome may display more severe symptoms. Therefore, the use of semen from non-carriers or sires of other families should be considered for the prevention of hereditary diseases when mating with dams that are either carriers of an *F11* and/or *CL-16* deficiency or members of a family of such carriers.

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Oligogenic transmission of abnormal teat patterning phenotype (ATPP) in cattle

N. Ihara*, T. Watanabe*, Y. Sato†, T. Itoh*,‡, T. Suzuki† and Y. Sugimoto*

*Shirakawa Institute of Animal Genetics, Odakura, Nishigo, Fukushima 961-8061, Japan. †Iwate Agricultural Research Center Animal Industry Research Institute Animal Biotechnology Research Section, Sunagomi, Takizawa, Iwate 020-0173, Japan.

Summary

Abnormal teat patterning phenotype (ATPP) is characterized by one (moderate form) or two (severe form) absent teats in cattle. Using an allele-sharing non-parametric linkage strategy, significant associations with severe ATPP animals were detected on BTA17 (Z_{max} = 7.3 at 21 cM), centromeric BTA1 (Z_{max} = 3.7 at 8 cM) and telomeric BTA1 (Z_{max} = 4.8 at 142 cM). The BTA17 region was also significantly associated in the analysis of the moderate ATPP animals (Z_{max} = 5.3 at 0 cM). The transmission disequilibrium test in severe ATPP animals demonstrated significant over-transmission of paternal alleles in the BTA17 region (P = 2.2×10^{-9}), the centromeric BTA1 region (P = 0.0351) and the telomeric BTA1 region (P = 0.005). Significant over-transmission of the BTA17 region was also observed among the moderate ATPP animals (P = 2.3×10^{-5}). These findings indicate that the BTA17 locus plays a key role in risk of the disease, and that the BTA17 locus contributes temporally in combination with the two other loci on BTA1 and/or possibly unknown modifier(s) in a probabilistic oligo- or polygenic manner of transmission. Haplotypes of these three loci can be used for marker assisted animal breeding to control the recurrence of affected progeny with ATPP.

Keywords absent teats, BTA1, BTA17, cattle, non Mendelian trait.

Introduction

Developmental defects manifesting abnormal teat patterning phenotype (ATPP) are reported in several species, including livestock animals (Howard & Gusterson 2000). These abnormal patterns include the presence of additional teats (supernumerary/hyperthelia), the absence of teats (athelia/hypothelia) and abnormal teat locations. In humans, some abnormalities are observed in clinically heterogeneous disorders such as Scalp-ear-nipple syndrome (OMIM 181270) and Ulnar-mammary syndrome (OMIM 181450) in which mutations in the *TBX3* gene, encoding T box transcription factor 3 (Tbx3), are suggested (Bamshad *et al.* 1997). In mice, five pairs of mammary glands are arranged in an ordered array along the ventral surface between the forelimbs and hindlimbs, and abnormal mammary patterning phenotypes, including absent teats, have been reported (Howard & Gusterson 2000).

In cattle, two pairs of teats are ventrally arranged inside the inguinal area in an ordered symmetrical pattern (Hurley & Ford 2002). Supernumerary teats are classically observed at a higher incidence in several dairy breeds, in which a genetic influence is suspected (Beka *et al.* 2002). As supernumerary teats are usually benign entities, the genetic basis and aetiologies are not well studied. On the other hand, absent teats are quite rare except for sporadic cases and, to our knowledge, there are few reports in cattle.

Here, we describe a rare ATPP with a high incidence (16%) of one or two absent teats within a Japanese Black cattle population. Residual teats function normally, and no other noticeable anomalies are observed in affected cattle. The aim of this study was to characterize the genetic nature of the ATPP with the goal of establishing a more sophisticated diagnostic system to control its incidence. This is the

Address for correspondence

T. Watanabe, Shirakawa Institute of Animal Genetics, Odakura, Nishigo, Fukushima 961-8061, Japan.
E-mail: toshiow@sage.or.jp
‡Present address: Livestock Improvement Association of Japan, Maebashi, Gunma 351-0121, Japan.

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first study in which the chromosomal region(s) responsible for developmental mammary defects, such as abnormal teat number, was determined.

Materials and methods

Animal samples and phenotypes

Assuming a correlation between the severity and number of missing teats, we used the Wilcoxon rank-sum test for interval mapping after transforming ATPP severity to rank statistics. This approach was used rather than transforming ATPP phenotypic subtypes, such as atrophy, one absent teat and two absent teats into numerical values and analysing the data using linear regression methods, in which paternal haplotype is regressed to a phenotypic value that is assumed to be normally distributed. We tentatively defined four phenotypic subsets of 606 half-sib progeny from a single bull: (i) 454 normal animals; (ii) 49 severe ATPP animals with two absent teats including 22 males and 27 females (Fig. S1a & d); (iii) 48 moderate ATPP animals with one absent teat including 23 males and 25 females (Fig. S1b); and (iv) 55 ATPP candidates including 38 males, 15 females and two sex-unknown with complete pairs of teats, but some teats atrophied, mislocated or a combination of these phenotypes (Fig. S1c).

Genome screen

DNA samples for the bull and 606 half-sibs were extracted from peripheral blood leucocytes using the automatic isolation system NA-1000 (Kurabo, Tokyo, Japan) according to the manufacturer's instructions.

In the first stage of the genome scan, the bull and 144 progeny comprising 91 normal (48 males, 43 females), 21 severe ATPP (8 males, 13 females), 19 moderate ATPP (12 males, seven females) and 13 ATPP candidates (seven males, four females and two sex-unknown subjects) were genotyped for 447 microsatellite markers selected from the Shirakawa-USDA linkage map (Ihara *et al.* 2004) with a 7 cM average interval covering the 29 bovine autosomes. Microsatellite markers used in the second scan are shown in Table S1. The progeny were increased to 606 animals for a total of 454 normal (245 males and 208 females), 49 severe ATPP (22 males and 27 females), 48 moderate ATPP (23 males and 25 females) and 55 ATPP candidates (38 males, 15 females and two sex-unknown subjects).

PCR conditions for microsatellite markers were optimized as described previously (Ihara *et al.* 2004). Microsatellite polymorphisms were analysed on an ABI 3700 DNA analyser (Applied Biosystems, Foster City, CA, USA). Genotype data were captured using GENESCAN 3.7 and GENOTYPER 2.1 software (Applied Biosystems).

Mapping procedure

To determine the map location for this disease locus, we used the Wilcoxon rank-sum test according to Kruglyak & Lander (1995). Briefly, the following statistic was defined:

$$Zw(s) = \frac{Yw(s)}{\sqrt{\{Vw(s)^2\}}},$$

where

$$Yw(s) = \sum_{i=1}^{n} [n + 1 - 2 \cdot rank(i)] \cdot P[g_i(s) | g_{i,L}, g_{i,R}]$$

in which n is the number of progeny; $rank(i)$ is the rank by phenotype of progeny i; $P[g_i(s) | g_{i,L}, g_{i,R}]$ is the probability that progeny i has the sire's first arbitrary haplotype transmitted at map position (s), given its genotype at the left ($g_{i,L}$) and right ($g_{i,R}$) flanking markers:

$$\sqrt{\{Vw(s)^2\}}$$

is the standard deviation of $\{w(s)\}$, expected under the null hypothesis of no linkage between phenotype and genotype over all possible sets of genotypes. Under the null hypothesis, Zw behaves asymptotically as a standard normal variable that generalizes to a non-parametric Wilcoxon rank-sum test at the marker position.

The phase of the sire's chromosomes was determined for each pair of two consecutive heterozygous markers using allele transmission to offspring information so that recombination between two markers was minimized. The analysis was performed at 1 cM intervals along the chromosome. For all analyses, marker locations were obtained from the Shirakawa-USDA linkage map (Ihara *et al.* 2004). Thresholds for significance of the Zw statistic value were obtained by 10 000 random permutations of the phenotypic data (Churchill & Doerge 1994). The highest Zw value over all analysis points for each analysis was recorded, and the 1% and 5% thresholds were defined as the 100th and 500th ranked Zw values. For chromosome-wise and experiment-wise thresholds, the highest statistical value on each chromosome and on all chromosomes was recorded.

Risk estimations

The transmission disequilibrium test by Fisher's exact test was used to identify significant deviations from the expected 1:1 Mendelian transmission in genetically assigned regions. The transmission frequency (τ) from bull to progeny was estimated from haplotype data supported by multiple microsatellite loci by maximum non-parametric linkage (NPL) scores (Z scores). Fisher's exact test with one degree of freedom based on the appropriate likelihood ratio was used to test whether $\tau = 1/2$. The risk ratio (RR) of each affected group within the family was calculated as follows:

$$RR = \frac{\text{frequency (\%) in the affected group}}{\text{frequency (\%) in the normal group}}$$

The incidence rate in each affected group was estimated from the frequency ratio in affected and normal groups as follows:

$$\text{Incidence rate} = \frac{\text{frequency (\%) in the affected group}}{\text{frequency (\%) in the affected group} + \text{frequency (\%) in the normal group}}$$

Results

Abnormal teat patterning phenotype affected animals (Fig. S1) did not display any anomalies other than teat patterning phenotypes. Reproduction in affected females appeared to be normal, as residual mammary glands normally undergo pubertal, pre- and post-pregnant mammary gland maturation and lactation in females with one absent teat. We subdivided the progeny into four groups based on the number of absent teats, similar to what was carried out with complex human diseases showing diverse phenotypes (Thornton-Wells *et al.* 2004). There was no significant difference in the incidence between severe (8%), moderate (8%) and ATPP candidates (9%). The sex ratio was almost even between severe and moderate ATPP groups, but there were 2.5-fold more male ATPP candidates than females ($P < 0.05$). Abnormal teat patterning phenotype seems to be incompatible with an autosomal single recessive condition, based on the incomplete phenotypic penetrance and pedigree records.

In this study, the bull and 144 progeny were genotyped for 447 microsatellite markers. Non-parametric linkage analyses of severe ATPP and normal progeny indicated that significant linkages were detected with paternal alleles of the centromeric markers on BTA17 ($Z_{max} = 5.0$ at 10 cM), based on a 1% experiment-wise threshold, and on BTA1 ($Z_{max} = 3.5$ at 139 cM) and BTA25 ($Z_{max} = 3.0$ at 49 cM), using 5% chromosome-wise thresholds. The same chromosomal region of BTA17 was also significant among the 19 moderate ATPP animals using a 5% chromosome threshold ($Z_{max} = 3.0$ at 0 cM). In the 13 ATPP candidates, significant linkage was detected on BTA7 ($Z_{max} = 3.8$ at 88 cM) using a 5% genome wise threshold.

In the second scan of BTA1, BTA7, BTA17 and BTA25, progeny numbers were increased to 616. As a result, the significance of the centromeric BTA17 region ($Z_{max} = 7.3$ at 21 cM) and the telomeric BTA1 region ($Z_{max} = 4.8$ at 142 cM) were increased to a 1% chromosome-wise threshold in the 49 severe ATPP animals (Fig. 1). An association with the severe ATPP form was detected in the centromeric region of BTA1 ($Z_{max} = 3.7$ at 8 cM). The BTA17 region was also significantly associated in the analysis of 48 of the moderate ATPP animals ($Z_{max} = 5.3$ at 2 cM) (Fig. 1). Two other regions on BTA25 and BTA7 respectively were no longer significant with the increased sample size. Thus, the 55 ATPP candidates were not analysed further.

Three chromosomal regions with 90% confidence intervals were chosen to estimate the RR and the incidence rates in progeny (Fig. 1): the 18-cM centromeric BTA17 region between *BMS499* (6 cM) and *BMS941* (24 cM); the 8 cM telomeric BTA1 region between *BMS918* (136 cM) and *DIK2050* (144 cM) and the 8-cM centromeric BTA1 region between *DIK4591* (2 cM) and *DIK4856* (10 cM). In the transmission disequilibrium tests between 47 severe ATPP and 395 normal animals, significant haplotype sharing was observed in the BTA17 region (Fisher's exact test, $P = 2.2 \times 10^{-6}$, RR = 2.2), the telomeric BTA1 region ($P = 0.005$, RR = 1.7) and the centromeric BTA1 region ($P = 0.035$, RR = 1.8) (Table 1). There was also strong over-transmission of paternal alleles in the BTA17 region in the moderate ATPP group ($P = 2.3 \times 10^{-3}$, RR = 1.9). Finally, we estimated the probabilities of ATPP based on the three loci haplotypes in progeny (Table 1). When progeny with all non-risk haplotypes of these three loci were selected, the estimated incidence rates were 0 for severe ATPP and 0.06 for severe or moderate ATPP forms (Table 1).

Discussion

The 18-cM centromere region on BTA17 with higher significance levels in both severe and moderate ATPP is homologous with human chromosome 4q28.3-q31.3, which covers a 14 Mb physical distance containing 59 known gene sequences (Itoh *et al.* 2005; UCSC: http://www.genome.ucsc.edu/). Mammary gland morphogenesis is dynamically regulated by a sequence of reciprocal signals between mesenchyme and epithelium at embryonic day 10 (E10) to E12.5 in mice (Parmar & Cunha 2004). Therefore, genes expressed in these embryonic layers in the corresponding stages at E32–E40 in cattle (Hurley & Ford 2002) are most likely to be functional candidates.

In the present study, we detected significant haplotype sharing through identical-by-descent (IBD) in three paternally transmitted chromosomal region haplotypes shared in moderate ATPP animals on BTA17, as well as two regions on BTA1. In addition, all severe ATPP animals with identified chromosome phases shared the 18-cM region on BTA17. This finding suggests that the locus on BTA17 has a vital role in the causality of both forms of ATPP. The affected haplotype on BTA17 in the bull was inherited from his father, while the two other affected haplotypes were from his mother.

These three loci, however, may not be sufficient to explain the genetics of ATPP. In fact, two moderate ATPP animals

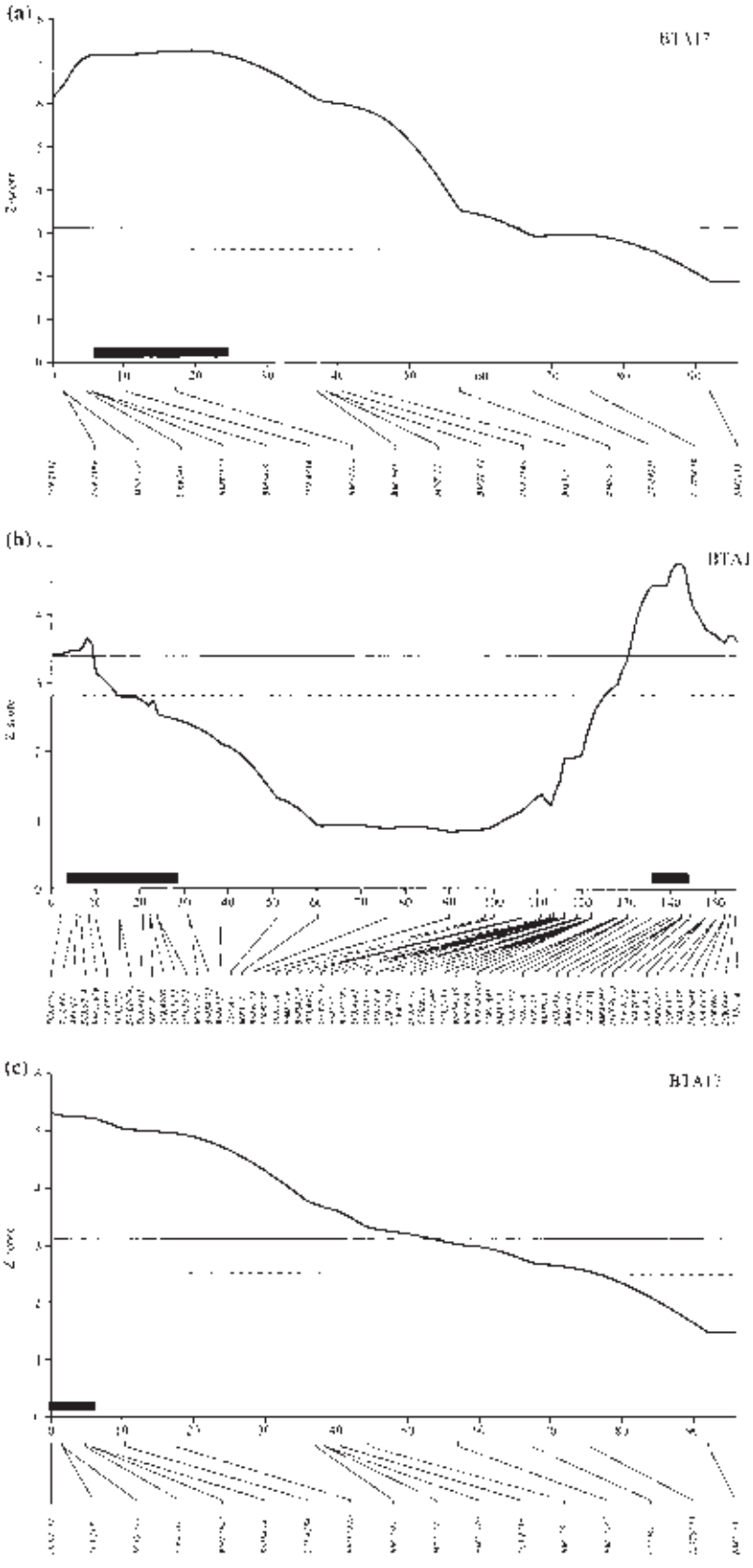

Figure 1 Non-parametric linkage profiles for ATFP in cattle on BTA17 and BTA1. Non-parametric linkage Z-score profiles of severe phenotype animals ($n = 49$) and normal animals ($n = 454$) on BTA17 (a) and BTA1 (b). A horizontal line and a dotted line indicate 1% and 5% chromosome-wise significance levels respectively. A black bar indicates the 90% confidence interval of the *ATFP* locus. (c) A Z-score profile of moderate phenotype animals ($n = 48$) and normal animals ($n = 454$) on BTA17.

Table 1 Risk/non-risk haplotypes distributed among normal and affected animals.

		Centromeric BTA17	Telomeric BTA7	Centromeric BTA1	A1	None
Normal (*n* = 395)	Risk haplotype (+)	46% (181)	48% (189)	42% (167)	9% (36)	–
	Non-risk haplotype (–)	54% (214)	52% (206)	57% (228)		17% (66)
	RR	1	1	1	1	1
	TDT (*P*-value)	NS	NS	0.03*	–	–
	Incidence rate	0.5	0.5	0.5	0.5	0.5
Severe (*n* = 47)	Risk haplotype (+)	100% (47)	79% (37)	74% (35)	57% (27)	–
	Non-risk haplotype (–)	0% (0)	21% (10)	26% (12)		0% (0)
	RR	2.2	1.7	1.8	6.3	
	TDT (*P*-value)	2.2×10^{-11}**	4.9×10^{-6}**	0.04*	–	–
	Incidence rate	0.68	0.62	0.64	0.86	0
Moderate (*n* = 45)	Risk haplotype (+)	87% (39)	38% (17)	51% (23)	18% (8)	–
	Non-risk haplotype (–)	13% (6)	62% (28)	49% (22)	–	4% (2)
	RR	1.9	0.8	1.2	2	–
	TDT (*P*-value)	2.3×10^{-7}**	NS	NS	–	–
	Incidence rate	0.65	0.44	0.55	0.66	0.12

RR, risk ratio; NS, not significant; TDT, transmission disequilibrium test
*$P < 0.05$, **$P < 0.001$ (Fisher's exact test)

(4%) did not have either of the risk haplotypes, and the normal bull and 36 normal progeny (9%) had the affected haplotypes (Table 1). These observations strongly support the existence of one or more additional modifier genes. The possibility of maternal intrauterine hormonal or nutritional environments affecting the condition cannot be excluded. It is unclear why affected individuals with all absent, three absent or two tandem absent teats were not observed. Genetically distinct signalling pathways might exist in mammary placode induction between front and rear teat pairs, as suggested in mice (Mailleux *et al.* 2002).

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Supplementary Materials

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2006.01543.x

Figure S1 Teat patterning phenotypes in affected animals.

Table S1 Informative microsatellite markers on BTA1, BTA7, BTA17 and BTA25.

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Mammalian Genome
Genes and Phenotypes

Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping

Akiko Takasuga,[1] Toshio Watanabe,[1] Yasushi Mizoguchi,[1] Takashi Hitano,[1] Naoya Ihara,[1] Atsushi Takano,[1] Kou Yokouchi,[1] Akira Fujikawa,[2] Kazuyoshi Chiba,[3] Naohiko Kobayashi,[4] Ken Tatsuda,[5] Toshiaki Oe,[6] Megumi Furukawa-Kuroiwa,[7] Atsuko Nishimura-Abe,[8] Tatsuo Fujita,[9] Kazuya Inoue,[10] Kazunori Mizoshita,[11] Atsushi Ogino,[12] Yoshikazu Sugimoto[1]

[1]Shirakawa Institute of Animal Genetics, Japan Livestock Technology Association, Odakura, Nishigo, Fukushima 961-8061, Japan
[2]Hokkaido Animal Research Center, Nishi, Shintoku, Hokkaido 081-0038, Japan
[3]Miyagi Prefectural Animal Industry Experiment Station, Iwadeyama, Osaki, Miyagi 989-6445, Japan
[4]Gifu Prefectural Livestock Research Institute, Kiyoma, Takayama, Gifu 506-0101, Japan
[5]Hyogo Prefectural Institute of Agriculture, Forestry & Fisheries, Kasai, Hyogo, 679-0103, Japan
[6]Tottori Animal Husbandry Experiment Station, Komura, Toushaku, Tottori 689-2503, Japan
[7]Okayama Prefectural Center for Animal Husbandry & Research, Misaki, Kume, Okayama 709-3494, Japan
[8]Shimane Prefectural Animal Husbandry Experiment Station, Koshi, Izumo, Simane 693-0043, Japan
[9]Oita Prefectural Institute of Animal Industry, Kuse, Takeda, Oita 878-0201, Japan
[10]Miyazaki Prefectural Livestock Experiment Station, Takahara, Nishimonokata, Miyazaki 889-4411, Japan
[11]Cattle Breeding Development Institute of Kagoshima Prefecture, Osumi, So, Kagoshima 899-8212, Japan
[12]Livestock Improvement Association of Japan, Inc., Machishin, Gimiya, 351-0124, Japan

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Abstract

To map quantitative trait loci (QTL) for growth and carcass traits in a purebred Japanese Black cattle population, we conducted multiple QTL analyses using 15 paternal half-sib families comprising 7860 offspring. We identified 40 QTL with significant linkages at false discovery rates of less than 0.1, which included 12 for intramuscular fat deposition called marbling and 12 for cold carcass weight or body weight. The QTL each explained 2% - 13% of the phenotypic variance. These QTL included many replications and shared hypothetical identical-by-descent (IBD) alleles. The QTL for CW on BTA14 was replicated in five families with significant linkages and in two families with a 1% chromosome-wise significance level. The seven sires shared a 1.1-Mb superior *Q* haplotype as a hypothetical IBD allele that corresponds to the critical region previously refined by linkage disequilibrium mapping. The QTL for marbling on BTA4 was replicated in two families with significant linkages. The QTL for marbling on BTA6, 7, 9, 10, 20, and 21 and the QTL for body weight on BTA6 were replicated with 1% and/or 5% chromosome-wise significance levels. There were shared IBD *Q* or *q* haplotypes in the marbling QTL on BTA4, 6, and 10. The allele substitution effect of these haplotypes ranged from 0.7 to 1.2, and an additive effect between the marbling QTL on BTA6 and 10 was observed in the family examined. The abundant and replicated QTL information will enhance the opportunities for positional cloning of causative genes for the quantitative traits and efficient breeding using marker-assisted selection.

Introduction

Quantitative trait loci (QTL) mapping studies have been performed extensively in many organisms; however, relatively few genes underlying genetically complex traits have been identified (Glazier et al. 2002). There are several problems in detecting QTL. One of the primary problems is the small effect of each contributing locus (Kruglyak 1996). Second, QTL interact with other genes and environmental

Correspondence to: Yoshikazu Sugimoto. E-mail: kazusugi@sing.or.jp

factors. Third, even QTL with significant linkages might be false positives as a result of unsuitable study design and/or the population studied. Finally, a limited number of offspring of the pedigree is an additional problem, particularly for domestic animal species such as cattle.

QTL mapping studies in beef cattle have been performed using experimental crosses between relatively different *Bos indicus* and *Bos taurus* breeds, and several significant QTL have been identified (e.g., Casas et al. 2000; Keele et al. 1999; Stone et al. 1999; the Bovine QTL Viewer (http://www.bovineqtl.tamu.edu/index.html)). The studies that examined the association between identical-by-descent (IBD) haplotypes and traits were followed up using commercial lines of *Bos taurus*; however, the phases of the associated haplotypes were different between the lines (Li et al. 2002), which resulted in difficulties narrowing down the QTL using this method.

On the other hand, several QTL studies in dairy cattle (Holstein breed) using a granddaughter design have reported multiple QTL for milk yield and composition, such as those on BTA6 (e.g., Georges et al. 1995; Spelman et al. 1996), BTA14 (Coppieters et al. 1998; Heyen et al. 1999), and BTA20 (Arranz et al. 1998; Georges et al. 1995). Furthermore, hypothetical IBD mapping and positional candidate cloning led to the successful identification of causal mutations in *ABCG2* on BTA6 (Cohen-Zinder et al. 2005) and/or *SPP1* on BTA6 (Schnabel et al. 2005), *DGAT1* on BTA14 (Grisart et al. 2002; Winter et al. 2002), and *GHR* on BTA20 (Blott et al. 2003), although relatively large haplotype blocks sometimes impede attempts to narrow down the critical region and identify the causal mutation (de Koning 2006). These identified mutations account for nearly 10% (*GHR F279Y* on protein %) to 44% (*OPN3907* indel on protein % adjusted for *DGAT1*) of the phenotypic variance (Blott et al. 2003; Schnabel et al. 2005). It could be important that a large number of Holstein cattle from a variety of local populations were employed for the analyses and that the mapped QTL were confirmed by replications.

To identify causative genes for beef cattle QTL, we collected more than 10,000 offspring, constructed 15 paternal half-sib families with more than 190 offspring, and searched for QTL replications as the first step. Recently, we confirmed that the progeny design simulated by Weller et al. (1990) and Moody et al. (1997) is useful for mapping QTL in purebred Japanese Black cattle populations (Mizoguchi et al. 2006; Mizoshita et al. 2004). Also, the use of a purebred local population minimizes the genetic background and environmental factors such as feeding, the fattening period, and the need for phenotypic assessment. The objectives of this study were to locate QTL for growth and carcass traits, confirm the QTL by replication, and identify the hypothetical IBD haplotypes harboring the QTL. We found 40 QTL with significant linkages, 15 of which were the replications of the QTL for carcass or body weight on BTA14, marbling on BTA4, and longissimus muscle area on BTA4 and 14. Another eight significant QTL were replicated with less than 5% chromosome-wise significance levels. Shared superior *Q* or inferior *q* haplotypes were observed in the QTL for carcass weight on BTA14 and for marbling on BTA4, 6, and 10. The identified haplotypes will provide useful information for identifying the causal genes as well as for marker-assisted selection in beef cattle.

Materials and methods

Simulation of the power to detect QTL. A simulation was performed to estimate the power to detect a QTL using a progeny design. A chromosome 105 cM long and with a single QTL residing at 52.5 cM was assumed. The eight markers that are all heterozygous in a sire were set to be located at 0, 15, 30, 45, 60, 75, 90, and 105 cM. The marker interval in the simulation (15 cM) was close to the average marker interval in this study (11.9 cM). A set of offspring was generated so that transmission of the QTL and the markers of the offspring occurred probabilistically according to the recombination fractions between the markers and the QTL. The expectation of the informative transmission ratio of paternal alleles was set to 0.6, which was close to the observed ratio in this study (0.625). A normal distribution of the trait values governed by the QTL was assumed. The normal distribution of the trait value variance was generated by an approximation of binomial distribution to normal distribution ($Bi(310, 0.5)$ or $Bi(330, 0.5)$). The simulation was performed for the QTL explaining 5% and 10% of the trait variance by allele substitution from *q* (inferior) to *Q* (superior), respectively, with increasing the number of offspring by intervals of 50 from 100 to 400. The QTL analyses were performed as described below. The simulation was repeated 1000 times and the number that exceeded either the 1% or 5% chromosome-wise significance level was counted.

Collection of DNA samples and phenotype data. Sixteen paternal half-sib families of Sire A through Sire O were constructed from carcass data and pedigree records collected by the Japan Wagyu Register Association (Kyoto, Japan). Two families

were constructed for Sire G, each of which consisted of steers and cows, respectively, and were collected from a different local area. The Sire N family and one Sire G family consisted of cows, while other families consisted of steers. Sire DNA was obtained from semen. Offspring DNA samples were collected from adipose tissues around the kidney at the slaughterhouses or from blood at the individual farmer's house. Six traits were analyzed in this study (Supplementary Table 1): body weight at slaughter (BW), cold carcass weight (CW), longissimus muscle area (LMA), rib thickness (RT, thickness of a muscle layer in a rib of beef), subcutaneous fat thickness (SFT), and marbling. Marbling was estimated using a beef marbling score (BMS) that ranks the carcass from 1 to 12 according to the degree of intramuscular fat deposition in which higher scores correspond to more marbling. These traits were systematically measured by certified graders and recorded at the slaughterhouses in Japan. Using farmers' data, the phenotypic values in Sire I and J families were corrected with best linear unbiased estimates of fixed effects estimated by MTDFREML (Boldman et al. 1995). Because offspring of Sire A and Sire C were collected from more than two slaughterhouses, their BMS values were corrected using the deviations

Genome screen. DNA was extracted from semen, blood, or adipose tissue according to standard protocols. The genome screen was conducted using the microsatellite markers on the Shirakawa-USDA linkage map (Ihara et al. 2004). The markers were selected individually for each sire as heterozygous for the sire and at an approximately 10-cM interval on autosomes. The average marker interval ranged from 8.0 (Sire D) to 16.0 cM (Sire C) in the families. Eleven families of Sire A through Sire J, consisting of 190 to 450 offspring, were individually genotyped, while four families of Sire K through Sire N, consisting of 320 to 760 offspring, were selectively genotyped using the 25%–50% extremes for the marbling trait (Supplementary Table 1). The genome screen for Sire O was done using less than 190 offspring and therefore is not presented. Polymerase chain reaction conditions were as described previously (Mizoshita et al. 2004). Polymerase chain reaction products were resolved by electrophoresis in polyacrylamide gels using an ABI 377 sequencer (Applied Biosystems, Foster City, CA) or an ABI 3700 DNA analyzer (Applied Biosystems). Genotype data were captured using GENESCAN and Genotyper software (Applied Biosystems).

Statistical analyses. Marker locations were obtained from the Shirakawa-USDA linkage map (Ihara et al. 2004). QTL analyses were performed with the interval mapping method using a linear regression model for half-sib families (Haley et al. 1994; Seaton et al. 2002), as described previously (Mizoshita et al. 2004). Briefly, phases of the sire's chromosomes were determined at each pair of two consecutive heterozygous markers using allele transmission information to offspring so that recombination between two markers was minimized. Linear regression analysis was performed using the following model:

$$y_i = \mu + \mathrm{Prob}(Q)_i a + e_i,$$

where y_i is the phenotypic value, μ is the fixed effect, a is the allele substitution effect from q to Q, $\mathrm{Prob}(Q)_i$ is the probability of Q phase at a given location, and e_i is the residual error for the ith individual. The analysis was performed at 2-cM intervals along a chromosome. To evaluate whether the QTL effect was well estimated, the information content (IC) was calculated as a variance of $\mathrm{Prob}(Q)_i$ divided by 0.25, the possible maximal variance of $\mathrm{Prob}(Q)_i$ (Knott et al. 1998). The allele substitution effect from q to Q was calculated as an estimator of a. The contribution ratio was calculated as a proportion of the trait variance explained by the paternal allele substitution from q to Q. Thresholds for significance of the F statistic value were obtained by 10,000 random permutations of the phenotypic data (Churchill and Doerge 1994). To control the error rate of multiple trait analysis, we applied the false discovery rate (FDR) proposed by Weller et al. (1998). The FDR was calculated at the marker-existing points in each family. The 95% confidence interval (CI) of the QTL locations was calculated by the bootstrapping method (Visscher et al. 1996).

Secondary screening. Secondary screening was performed for a total of 31 chromosomes of 12 sires (Sires A, C–F, G (cows), H, I, K, M–O). The families selectively genotyped in the genome scanning (Sires K, M, and N) were individually genotyped and calculated for all traits. Other families were individually genotyped using an increased number of offspring, resulting in 300–872 offspring per family, and calculated for all traits. From 7 to 47 markers were used per chromosome.

Results and discussion

Simulation and experimental framework. **Number of offspring.** The progeny design can detect only QTL with moderate to large effects (Moody et al. 1997). To determine how many offspring should be used for the analysis, we simulated the power of detecting a QTL (Fig. 1). The probability of detecting QTL accounting for 5% and 10% of the trait variance

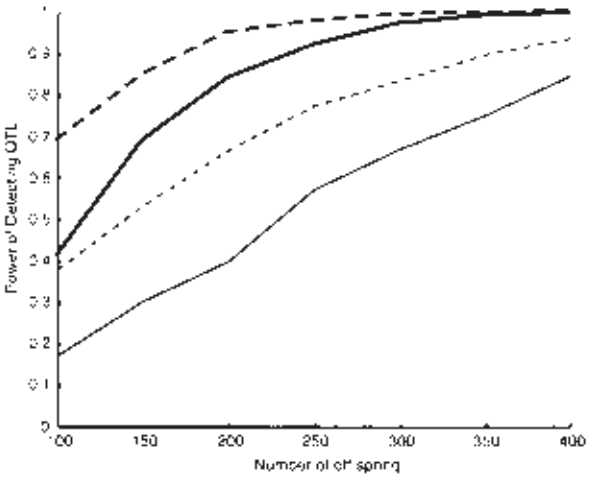


Fig. 1. Simulation of the power of detecting a QTL. The power of detecting a QTL explaining 5% (thin lines) or 10% (bold lines) of the trait variance was estimated. The broken and the solid lines indicate the power of detecting a QTL at 5% and 1% chromosome-wise significance levels, respectively.

was 0.66 and 0.95, respectively, at the 5% chromosome wise significance level using 200 offspring, although this threshold might produce one or two false positives as type I errors in a whole-genome scan. The power of detecting a QTL accounting for 5% of the trait variance reached 0.94 by increasing the number of offspring to 400 under the same conditions. In this study, we used 15 families whose sires were produced by line breeding or line cross (Fig. 2). Eleven families, consisting of 190–450 offspring, were individually genotyped and analyzed for growth and carcass traits, and in the remaining four families, consisting of 320–760 offspring, the 25%–50% extremes were selectively genotyped for the marbling trait only (Supplementary Table 1). According to Lander and Botstein (1989), the power obtained from the genotyping of the 33% extremes (greater than 1.5*D* from the mean) in a 25% larger population is almost equivalent to the power obtained from individual genotyping of the original population size. The simulation suggested that a QTL accounting for 5% of the trait variance could be detected at more than 66% probability in our study.

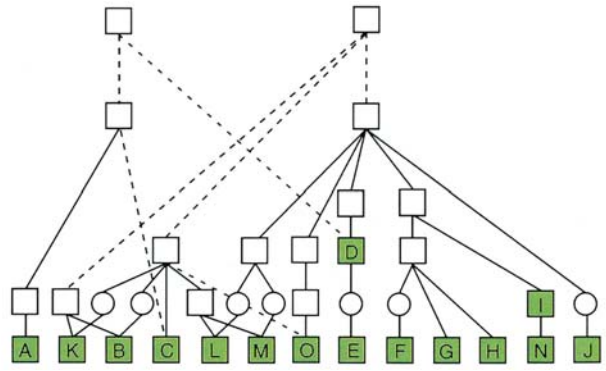


Fig. 2. Pedigrees of the sires used in this study. A dotted line indicates a distant relationship with more than two generations.

Selection of markers. In general, the average heterozygosity of microsatellite markers in the Japanese Black cattle population was almost the same as that in Holstein and slightly lower than that in the MARC reference population, which includes *Bos indicus* (Bishop et al. 1994) (0.71 in Japanese Black (N = 96); 0.73 in Holstein (N = 96); 0.79 in the MARC reference population; 78 microsatellites examined (data not shown)). To QTL analyze the 15 half-sib families, we selected heterozygous microsatellites for each sire at approximately 10-cM intervals. The average marker interval in the family ranged from 8.0 (Sire D) to 16.0 cM (Sire C). The overall marker interval averaged 12 cM, ranging from 3.3 to 72 cM. Ninety-five percent of the intervals were less than 27 cM and the remaining intervals lacked any available heterozygous markers. The average heterozygosity of the markers in the family ranged from 0.61 (Sire I) to 0.71 (Sire A). The heterozygosity of the markers in detected QTL regions was comparable to that in other regions (data not shown). The ratio of identified paternal alleles was 0.62 (589,066 among 945,561 total genotypes), ranging from 0.45 (Sire B) to 0.70 (Sire D) in the families, which assured correct determination of the phase of the sire's chromosomes.

Correlation between traits. Six traits were analyzed in this study: BW, CW, LMA, RT, SFT, and marbling. Phenotypic data for carcass traits of each half-sib family are available in Supplementary Table 1. Pairwise correlation coefficients between the traits were calculated per family (Supplementary Table 2). CW was highly correlated with BW (r = 0.96) and moderately correlated with RT (r = 0.59). Interestingly, there was a weak to moderate correlation between LMA and both CW and beef marbling score (BMS; r = 0.43), whereas the correlation between CW and BMS varied among families (r = 0.06–0.39). BMS and SFT were not correlated (r = 0.03).

Overall QTL analyses. Table 1 summarizes the QTL detected in genome scanning. Eleven families of Sire A through Sire J were analyzed for five or six traits and the remaining four families of Sire K through Sire N were selectively genotyped and ana-

Table 1. Summary of the QTL with significant linkages detected in genome scanning

Half-sib family	*Number of offspring*	*BTA*	*Number of markers*	*Average IC*	*Trait*	*Position (cM)*	*F statistic*	*IC on the position*	*Q to q allele substitution effect*[a]	*Contribution ratio (%)*	*Chromosome-wise significance level*[b]	*Experiment-wise significance level*[c]	*LOD*
Sire A	372	9	37	0.84	BMS	36	29.3	0.90	1.1	7.1	***	@@@	0.000
		28	6	0.83	SFT	56	14.6	0.80	0.3	3.5	***	@	0.026
Sire B	328	20	11	0.76	BMS	52	16.6	0.90	1.0	4.6	***	@	0.000
Sire D[d]	236	1	17	0.83	BW	76	16.3	0.83	29.4	6.1	**	@	0.000
		4	17	0.84	LMA	64	15.4	0.89	4.0	5.8	**	@	0.000
		4			BMS	74	17.0	0.96	1.1	6.4	***	@	0.008
		14	18	0.86	BW	46	28.8	0.92	36.3	10.6	***	@@@	0.000
		14			CW	46	28.1	0.92	25.4	10.3	***	@@@	0.000
Sire E	192	14	31	0.86	LMA	68	15.9	0.94	3.8	7.2	**	@	0.090
Sire F	246	2	9	0.79	SFT	30	16.1	0.74	0.5	5.8	**	@	0.000
		7	10	0.79	BW	80	14.8	0.98	21.4	5.3	**	@	0.056
Sire G	379	12	9	0.52	SFT	108	14.1	0.60	0.4	4.2	**	@	0.043
(steers)		14	15	0.83	LMA	44	15.4	0.91	3.8	4.6	***	@	0.000
		14			CW	38	15.1	0.85	22.3	4.5	**	@	0.032
Sire H	353	3	5	0.52	RT	64	15.4	0.81	0.4	3.9	***	@	0.036
		10	6	0.56	BW	50	16.0	0.61	34.4	4.1	***	@@	0.000
		10			CW	48	13.8	0.66	20.8	3.5	**	@	0.000
Sire I[e]	282	13	6	0.67	SFT	28	13.5	0.85	0.3	4.6	***	@	0.062
		14	11	0.67	CW	26	14.0	0.76	16.0	4.8	**	@	0.068
		21	11	0.76	BMS	78	20.9	0.88	1.1	7.2	***	@@	0.000
Sire J	258	2	10	0.77	LMA	52	15.1	0.78	3.6	6.0	**	@	0.030
		14	15	0.72	CW	48	43.6	0.82	26.1	12.9	***	@@@	0.000
		14			LMA	56	26.9	0.79	4.7	10.5	***	@@@	0.000
Sire K	158[g] 524[f]	14	11	0.73	BMS	22	19.9	0.61	NC[h]	NC[h]	***	@@	NC[h]
Sire N	190[g] 760[f]	6	10	0.76	BMS	84	15.5	0.82	NC[h]	NC[h]	***	@	NC[h]
		10	9	0.75	BMS	72	25.9	0.88	NC[h]	NC[h]	***	@@@	NC[h]

The QTL that did not exceed 5% experiment-wise significance level are shown in Supplementary Table 4
[a]The unit is kg for BW and CW, cm^2 for LMA, and cm for RT and SFT, respectively.
[b]*, **, and *** mean <5%, <1%, and <0.1% chromosome-wise significance levels, respectively
[c]@, @@, and @@@ mean <5%, <1%, and <0.1% experiment-wise significance levels, respectively.
[d]Mizoshita et al. 2004
[e]Mizoguchi et al. 2006
[h]NC: not calculated for the selectively genotyped families.
[g]The number of the offspring selectively genotyped.
[f]Total number of the offspring.

lyzed for marbling (Supplementary Table 1). In the 11 families, a total of 200 regions reached the 5% chromosome-wise significance level. To control for type I errors due to multiple tests for multiple traits, we calculated the FDR for each region (Weller et al. 1998). One hundred forty-three regions failed to pass the FDR threshold (< 0.11) (Weller et al. 1998), which comprised one third of the regions detected at a less than 1% chromosome-wise significance level (23/74) and most of the regions detected at the 5% chromosome-wise significance level (120/126) (Supplementary Table 3). These regions were eliminated from the study, although some of them might be real because FDRs were calculated irrespective of the phenotype correlation. For the four selectively genotyped families, 24 regions were detected at a less than 5% chromosome-wise significance level. In total, 81 regions were detected as provisional QTL (Supplementary Table 4). Each QTL explained 2.2% - 12.9% of the phenotypic variance in the family, as expected from the simulated data (Fig. 1). Among them, six QTL were highly significant (<0.1% experiment-wise significance level) and 20 were significant (<5% experiment-wise significance level) according to the criteria formulated by Lander and Kruglyak (1995) (Table 1).

Based on the genome scanning results, secondary screening was performed for a total of 31 chromosomes of 12 sires with increased numbers of offspring and markers. We detected 65 QTL at a less than 5% chromosome-wise significance level, including 40 that were not detected as provisional QTL in genome scanning (Table 2; Supplementary Table 5). On the other hand, one QTL that was detected at the 1% chromosome-wise significance level by selective genotyping did not reach the 5% chromosome-wise significance level after individual genotyping (Supplementary Table 4). Fourteen QTL newly exceeded 0.1% chromosome-wise significance level and therefore we regarded them as significant QTL (Table 2). Finally, we obtained 40 QTL with highly significant or significant linkages composed of 12 for marbling, 7 for CW, 5 for BW, 11 for LMA, 4 for SFT, and 1 for RT, and 80 other provisional QTL.

QTL locations with a 95% CI are shown in Fig. 3. Highly significant or significant QTL were distributed on 15 chromosomes (BTA1–4, 6–10, 12–14, 20, 21, and 28), and other provisional QTL were distributed on 25 chromosomes. As expected from the correlation between the traits, the QTL for CW were also detected for BW, and vice versa for the QTL for BW. Also, some of the QTL detected for LMA overlapped with the QTL detected for CW or marbling.

Several QTL were replicated. Significant linkages for marbling and LMA on BTA4 were detected in Sire D and Sire I families, and for CW and LMA on BTA14 were five families, respectively (Sires D, G, I, J, and N for CW and Sires E, G, J, K and O for LMA). In addition, the significant QTL for marbling on BTA6, 7, 9, 10, 20, and 21 and for BW on BTA6 were replicated at a 1% or 5% chromosome-wise significance level among families. The replicate results supported the presence of the QTL as well as the reliability of the analyses. These QTL are expected to be effective independent of the genetic background because they were detected in various local populations. Next, we further examined the replicated QTL for CW, BW, and marbling.

QTL for CW and BW. Twelve highly significant or significant QTL were detected for BW or CW, which were distributed on BTA1, 6, 7, 10, and 14 (Tables 1 and 2; Fig. 3). Thirteen other regions were detected at a less than 5% chromosome-wise significance level (Supplementary Tables 4 and 5). The QTL on BTA6 and 14 were replicated in different pedigrees as described above.

The *CW-1* QTL was originally mapped to BTA14 using the Sire D family and its location was narrowed down to the 1.1-Mb region by linkage disequilibrium mapping (Mizoshita et al. 2004, 2005). Highly significant or significant linkages for CW on BTA14 were detected in four additional families (families from Sires G, I, J, and N) and suggestive linkages were detected in two families (families of Sires K and O; Tables 1 and 2; Supplementary Tables 4 and 5). Because the QTL analyses were individually performed, in which different markers were used among the analyses (Fig. 4A), the haplotypes of the sires were examined more precisely around the *CW-1* region (41.7–49.8 cM) by adding more microsatellite markers. The superior *Q* and inferior *q* haplotypes of the seven sires were classified into two (*Q1* and *Q2*) and three haplotypes (*q1*, *q2*, and *q3*), respectively, and *Q1* and *Q2* shared a common haplotype between DIK7013 and NRKM-040, which corresponds to the critical region refined by the linkage disequilibrium mapping (Mizoshita et al. 2005) (Fig. 4B). The fact that many sires harbored *CW-1Q* suggested that this QTL was already successfully selected. The *Q* frequency in Japanese Black cattle was estimated to be nearly 50% (Mizoshita et al. 2005), which means that this QTL is still important for increasing CW. Also, the relatively high frequency suggests that the QTL has no apparent disadvantage for animal health or for the economically important carcass traits analyzed in this study. Interestingly, marbling and/or LMA QTL, with significant or suggestive linkages, were concomitantly mapped around the *CW-1* locus (Fig. 3), at which each superior (*Q*) allele resided in

Table 2. Summary of the QTL with significant linkages detected in secondary screening

Half-sib family	*Number of offspring*	*BTA*	*Number of markers*	*Average IC*	*Trait*	*Position (cM)*	*F statistic*	*IC on the position*	*Q to q allele substitution effect*[a]	*Contribution ratio (%)*	*Chromosome wise significance level*[b]
Sire A	785[c]	9	47	0.87	BMS	40	38.1	0.98	0.8	4.5	***
		9			LMA	38	24.3	0.99	2.5	2.9	***
Sire C	563[d]	21	18	0.68	BMS	40	16.8	0.89	1.1	2.7	***[h]
Sire D[e]	384	4	32	0.89	BMS	56	33.5	0.98	1.2	7.8	***
		4			LMA	60	19.0	0.94	3.4	4.5	***
	348	14	17	0.82	BW	50	45.9	0.92	39.6	11.5	***
		14			CW	50	40.0	0.92	26.7	10.6	***
Sire E	393	6	32	0.83	BW	42	29.9	0.88	41.7	6.9	***
		6			CW	38	24.7	0.90	24.1	5.7	***[h]
Sire F	496	7	16	0.84	BMS	28	28.0	0.93	1.0	5.2	***
					BW	82	14.9	0.92	16.0	2.7	**
Sire H	410	10	23	0.80	BW	52	13.0	0.70	26.5	2.9	**
		10			CW	44	11.5	0.96	14.3	2.5	**
Sire I[f]	872	4	14	0.61	BMS	66	56.7	0.90	0.9	5.9	***[h]
		4			LMA	42	15.1	0.79	1.9	1.6	***[h]
		14	12	0.73	CW	36	55.0	0.70	18.2	5.8	***
		14			LMA	24	29.6	0.75	2.7	3.2	***[h]
		21	35	0.78	BMS	80	18.0	0.96	0.5	1.9	***
Sire K	534	9	28	0.88	BMS	80	20.6	0.92	0.8	3.6	***
		14	20	0.88	BMS	38	17.1	0.89	0.7	3.0	***
		14			LMA	48	14.9	0.89	2.6	2.6	***[i]
Sire N	760	6	45	0.71	BMS	94	21.2	0.95	0.7	2.6	***
		10	25	0.73	BMS	78	27.6	0.98	0.8	3.4	***
		14	25	0.80	CW	44	26.0	0.95	15.4	3.2	***[i]
		14			LMA	30	16.5	0.89	3.1	2.0	***[i]
Sire O[g]	300	8	28	0.86	BMS	4	22.7	0.83	1.0	6.8	***
		14	28	0.86	LMA	72	23.7	0.88	3.9	7.1	***

Provisional QTL detected in chromosome scanning are shown in Supplementary Table 6.
[a]The unit is kg for BW and CW, cm^2 for LMA, and cm for RT and SFT, respectively.
[b]*, **, and *** mean <5%, <1%, and <0.1% chromosome-wise significance levels, respectively.
[c]Three hundred forty-eight steers from Market 1 and 437 from Market 4.
[d]Three hundred twenty-five steers from Market 1, 143 from Market 2, and 95 from Market 3.
[e]Mizoshita et al. 2004.
[f]Muroguchi et al. 2006.
[g]Genome scanning was done using less than 190 offspring and therefore was not included in Table 1.
[h]The QTL was FDR≥0.1 or not detected at 5% chromosome-wise significance level in genome scanning.
[i]The trait was not examined in genome scanning because of selective genotyping for BMS.

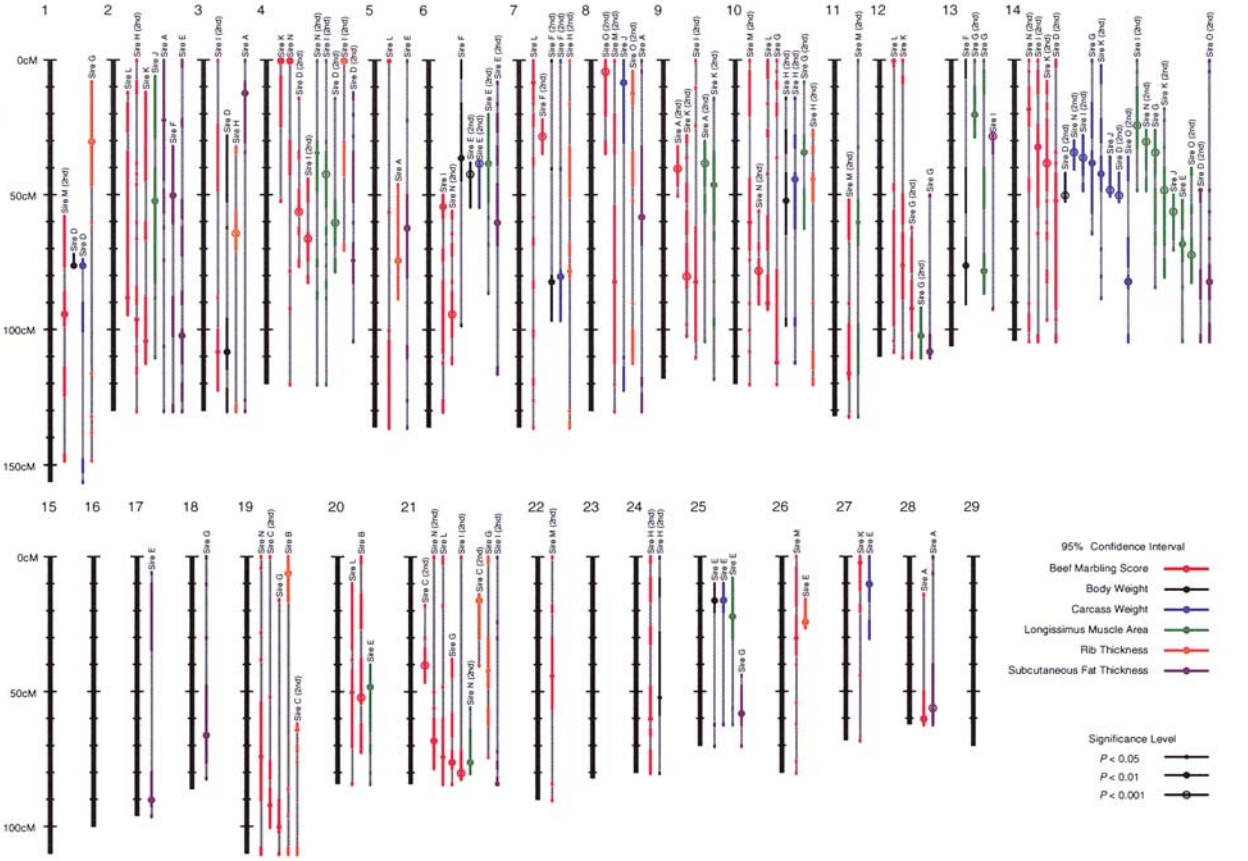


Fig. 3. Bovine QTL map. The QTL detected at less than 5% chromosome-wise significance level with less than 0.1 false discovery rate (FDR) are shown with 95% CI. For the QTL submitted to chromosome scanning, 95% CI of the fine-mapped regions are shown.

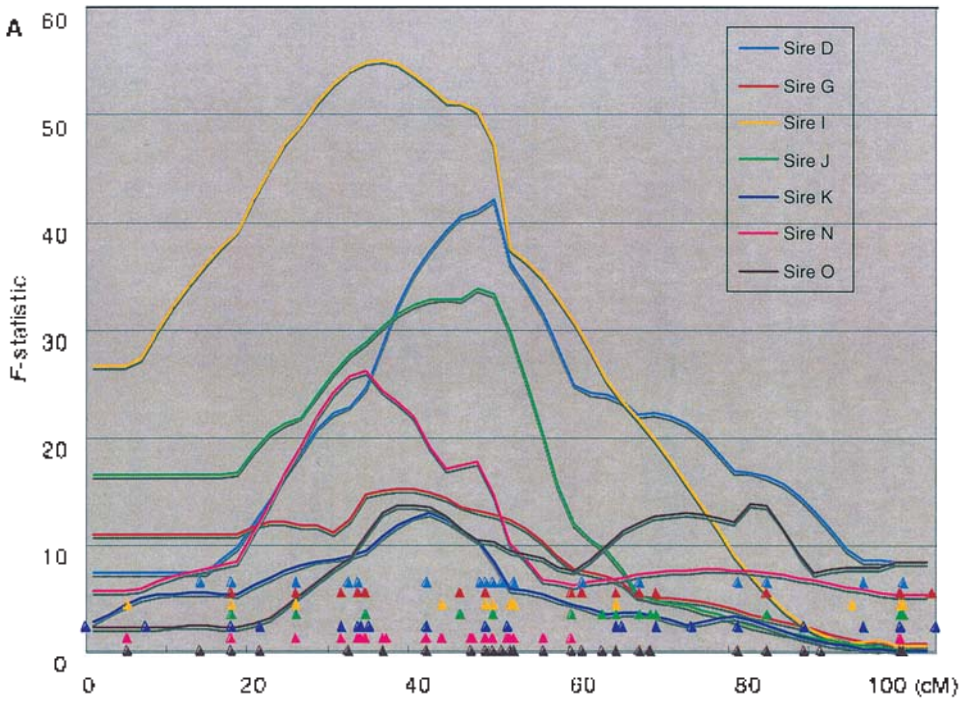
the same haplotype as the *CW-1 Q* allele, except for the marbling QTL detected at the 5% chromosome-wise significance level in the Sire D family (data not shown). It is likely that the selection for CW acts mainly at the *CW-1* locus because of a possible hitch-hiking effect on the marbling and/or LMA QTL.

The *Q* to *q* allele substitution effect of *CW-1* ranged from 13.7 to 26.7 kg in the seven families (Tables 1 and 2), which is comparable with the effect examined using 1700 steers: 23.6 kg for the first *Q* and an additional 15.2 kg for the second *Q* (Mizoshita et al. 2005). Interestingly, the average CW of the family differed by 60.5 kg (95%CI: 54.4 – 66.6 kg) between the Sire K and Sire J families (Supplementary Table 1). The difference was bigger than the effect of the *CW-1* locus, which should be due to the result of other CW QTL rather than a difference in the maternal *CW-1 Q* frequencies in the local populations.

The BW QTL on BTA6 were detected at the 0.1% and 1% chromosome wise significance levels in the Sire E and Sire F families, respectively (Table 2, Supplementary Table 4). This QTL, designated *CW-2*, was fine-mapped between 38 and 54 cM in the Sire E family (Fig. 3). There were no apparent shared haplotypes between Sire E and Sire F in the region, although they harbored common *Q* alleles for some markers (data not shown). The allele substitution effect on BW was 41.7 kg (Sire E) and 26.5 kg (Sire F), respectively, which corresponded to 24.1 kg and 13.4 kg on CW, respectively (Tables 1 and 2, Supplementary Table 4). Therefore, the effect of *CW-2* was nearly equal to that of *CW-1*. Because Sire E harbored a homozygous *Q* in the *CW-1* region (Fig. 4B), *CW-2* is expected to act additively on *CW-1*.

The QTL for carcass yield and/or growth rate on BTA1, 6, 10, and 14 were previously mapped using experimental crosses (Bovine QTL Viewer, http://www.bovineqtl.tamu.edu/index.html). Searching the *Q* haplotypes in other breeds might be useful if they have common founders. In humans and mice, many genes affecting body weight have been identified (Mouse Genome Informatics, http://www.informatics.jax.org/). In the human, the region

A



B

	Marker	BMS1941	DIK7008	DIK7012	DIK7013	DIK7015	DIK7018	NRKM-040	DIK7020	BM4630	DIK519	DIK062
	cM	41.707						47.116		45.799	48.925	49.828
Sire D	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire O	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire K	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire I	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q2	3	1	2	3	2	1	1	4	2	3	2
Sire N	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q2	3	1	2	3	2	1	1	4	2	3	2
Sire J	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q3	1	1	2	1	1	2	2	1	1	3	2
Sire G	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q3	1	1	2	1	1	2	2	1	1	3	2
Sire E	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	Q1	2	1	1	3	3	2	3	5	1	1	2

CW-1, 1.1-Mb region

Fig. 4. (A) *F*-statistic profiles for CW on BTA14. Marker locations are indicated by triangles colored for each family. (B) Haplotype analysis around the *CW-1* region. The microsatellite markers are ordered according to the physical map described in Mizoshita et al. (2005). The superior *Q* and inferior *q* haplotypes of the seven sires are grouped into two (*Q1* and *Q2*) and three (*q1*, *q2*, and *q3*) haplotypes, respectively.

corresponding to *CW-1* (HSA 8q12.1–12.2) contains no apparent candidate genes (Itoh et al. 2005; Mizoshita et al. 2005). Identification of the *CW-1* gene and other CW QTL genes might elucidate a novel molecular mechanism underlying growth and meat quantity in mammals.

QTL for marbling. Marbling is ranked according to the degree of intramuscular fat deposition and is highly related to beef quality. We mapped 12 significant and 33 provisional QTL for marbling (Tables 1 and 2, Supplementary Tables 4 and 5). The significant QTL were distributed on BTA4, 6,

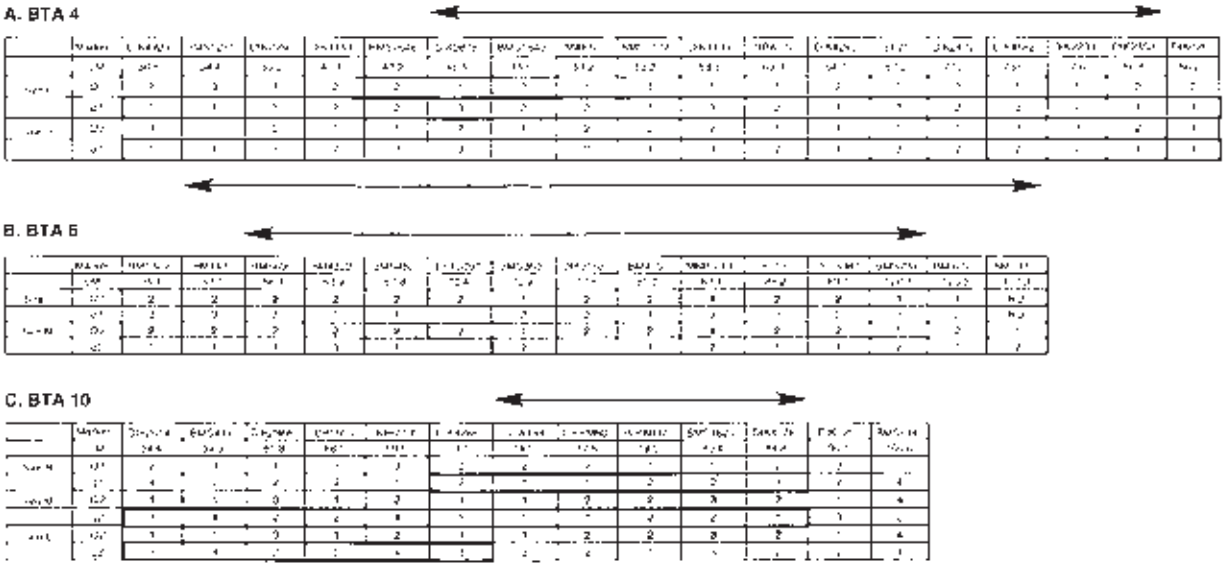


Fig. 5. Haplotype analyses for the replicated marbling QTL regions. (A) BTA4, (B) BTA6, (C) BTA10. The arrows indicate 95% CI of the QTL with significant linkages. Shared *Q* and *q* haplotypes were shown by grayed and bold lined boxes, respectively.

7, 8, 9, 10, 14, 20, and 21, which were replicated at a less than 5% chromosome-wise significance level except for the QTL on BTA8 and 14 (Fig. 3). These haplotypes in the replicated regions were compared between sires using the microsatellites on the Shirakawa-USDA linkage map (Ihara et al. 2004). In BTA6, an IBD superior *Q* haplotype was inherited from Sire I to Sire N (Figs. 2 and 5B). In BTA10, Sire I and Sire M shared an IBD *Q* haplotype inherited from a common sire, whereas no apparent shared *Q* haplotype was observed between the two sires and Sire N (Figs. 2 and 5C). Alternatively, Sire N shared an inferior *q* haplotype with Sire M in the QTL region. Also in BTA4, an IBD inferior *q* haplotype was shared between Sire D and Sire I (Fig. 5A). On the other hand, there was no significant shared haplotype for either *Q* or *q* in other QTL regions, although the QTL peaks in BTA9 and 20 were too broad to compare the haplotypes in detail (data not shown). Because the haplotype blocks of the QTL regions might be smaller than those examined, more detailed comparisons might reveal a shared *Q* haplotype.

The allele substitution effect of the significant marbling QTL ranged from 0.5 to 1.2 (Tables 1 and 2). Those of shared alleles were 0.9–1.2 (BTA4), 0.7–0.9 (BTA6), and 0.7–0.8 (BTA10), respectively (Supplementary Tables 4 and 5). The regression analyses suggested that the QTL on BTA4 and 21, BTA9 and 14, and BTA6 and 10 were additive in Sire I, Sire K, and Sire N families, respectively (Mizoguchi et al. 2006; Supplementary Table 6). The size of the effect and the interaction among the loci should be further examined in a general population.

It has been shown that linkage disequilibrium mapping is a useful method to narrow down the QTL regions (Farnir et al. 2002; Mizoshita et al. 2005). We expect it is also workable for replicated QTL using a general population collected throughout Japan. On the other hand, the *Q* haplotypes of some marbling QTL were inherited in maternal lineages, and their frequencies were too low in the population to examine the association between the alleles and marbling (data not shown). The QTL analyses of descendant sires might be useful to confirm and narrow down the QTL.

Our investigation outlined marbling QTL in Japanese Black cattle. The QTL for marbling score or meat tenderness have been mapped on several chromosomes using crossbred populations (Bovine QTL Viewer, http://www.bovineqtl.tamu.edu/index.html), while QTL on BTA4, 7, 8, and 21 were not detected previously. The ability to deposit intramuscular fat that accounts for high marbling might be a characteristic of Japanese Black cattle, and the *Q* allele frequencies of these QTL might be low in other breeds.

The genes involved in adipogenesis have been studied extensively. More than 1000 genes are transcriptionally regulated in adipogenesis (Soukas et al. 2001; Tseng et al. 2005). In cattle, approximately 80 genes have been isolated whose expression levels are different between high-marbled Japanese Black and low marbled Holstein biopsied

muscle (Sasaki et al. 2006). Physical assignment of these genes might help us find a candidate gene for mapped QTL (Yamada et al. 2006). So far, the transgenic mouse overexpressing ADAM 12 under the muscle creatine kinase promoter is the only animal model in which intramuscular fat is deposited in skeletal muscle (Kawaguchi et al. 2002). Identification of the marbling QTL genes will provide new insight into adipogenesis.

Perspectives. We detected 40 QTL for growth and carcass traits with moderate to large effects using 15 paternal half-sib families of purebred Japanese Black cattle, and we further confirmed their presence by replication and IBD mapping. This QTL information will be applied to breed descendant sires, followed by confirmation of the effects using the resulting half-sib family. Some QTL regions detected in this study were overlapped with the QTL detected using crossbred populations in previous reports. Our QTL results using a purebred Japanese Black population might be applicable to other bovine breeds. Recently, the bovine SNP consortium produced more than 120,000 SNP, and the 10k SNP array is commercially available (Affymetrix, Santa Clara, CA). Genome-wide linkage disequilibrium mapping is now possible using the array; it might be useful to examine the size of the effect and narrow down the region of each QTL.

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〔ミニレビュー〕

ウシゲノム解析用ツールの開発とゲノム解読の現状

高須賀晶子

（社）畜産技術協会附属動物遺伝研究所

Development of bovine genomic tools and the progress of the bovine genome sequencing project

Akiko TAKASUGA

Shin-Kawa Institute of Animal Genetics

1. はじめに

ゲノム解析のツールとして，連鎖地図，RH 地図，BAC 整列地図など（後に詳述する）があげられるが，究極は全ゲノム塩基配列の決定である．2001 年にヒトゲノムドラフト配列が決定されて以降，哺乳動物としては，マウス（2002 年），ラット（2004 年），チンパンジー（2005 年），イヌ（2005 年）のゲノム配列が決定され，次いで，今，ウシのゲノム配列が決定されつつある．家畜の中では，ニワトリに次ぐ二番手となり，三番手にはブタの配列決定が予定されている．ウシのゲノム塩基配列決定は，米国テキサス大学ベイラー校のヒトゲノムシークエンシングセンターで行われているが，ここは，一ヶ月で一つの哺乳動物種ゲノムの塩基配列を読む力を持っているそうだ．が，実際は，最初にあげたような地図（それも，多数のマーカーを持つ高密度のもの）がなければ，染色体上の位置のわからない，短い配列断片が大量にできるだけで，ゲノム配列にはならない．

動物遺伝研究所では，ウシゲノムプロジェクトが始まる以前から，研究を効率良く進めるにはゲノム解析用ツールの充実が必須と考え，米国農務省肉畜研究センター（USDA-MARC）と共同で高密度連鎖地図の作成に，また，ネバダ大との共同で高密度 RH 地図の作成に着手した．その後，米国を中心とした国際コンソシアム（日本の研究機関は参加していない）が結成され，BAC フィンガープリント整列地図などが作成されつつある．ウシゲノム塩基配列については，2004 年 9 月に，3 × WGS（Whole Genome Shotgun）配列（全ゲノムを断片化した配列を，のべで全ゲノムの 3 倍に相当する長さになるまで読んだもの）が，ついで，2005 年 3 月に，6 × WGS 配列が公開された．ここでは，私たちの作成した高密度連鎖地図および RH 地図を，ウシゲノム解読の現状とともに紹介したい．また，ゲノム解析に詳しくない方のために，次項で各地図の特長と役割について記したので，参照していただければと思う．

2. 各々の地図の特徴と役割

地図には，連鎖地図と物理地図（RH 地図や BAC 整列地図）とがあり，連鎖地図は，マーカー間の距離を遺伝的な物差し（連鎖の度合い，すなわち遺伝上の組み換え頻度）で測り，並べたものであるのに対して，物理地図は DNA の長さという物理的な物差しの上にマーカーを並べたものである．

(1) 連鎖地図

減数分裂の過程において，相同染色体間の組み換えが起こることが知られているが，その際，ある 2 マーカーの位置（座位という）が同一染色体上で近くにあればあるほど，その間で組み換えの起こる確率は低くなり，ともに親から子に受け継がれる（連鎖する）確率が高くなる．一方，距離が遠ければ，その間で組み換えの起こる確率は高く，連鎖しない．多数の減数分裂が観察できる大きな家系で，各マーカーの伝わり方を調べると，マーカー間の組み換え頻度が算定でき，遺伝的に適当にマーカーを並べることができる．

連鎖地図のマーカーには，相同染色体間を識別でき

連絡先：高須賀晶子，（社）畜産技術協会・附属動物遺伝研究所
〒 961-8061 福島県西白河郡西郷村大字小田倉字小田倉原
e-mail takasuga@sirg.or.jp

る多型性が必要である．ゲノム上には，マイクロサテライトと呼ばれる短い塩基配列の繰り返しから成る反復配列（$(CA)_n$ など）が散在しており，その繰り返し数 n は各相同染色体に固有であるので，連鎖地図のマーカーとして汎用される．

連鎖地図の役割には，大きく2つがあり，ひとつは，遺伝病や経済形質などの座位をマッピングするためのマーカーの提供，もう一つは，物理地図作成のための足場の提供である．

(2) RH 地図

RH 地図は，放射線照射体細胞雑種（Radiation Hybrid）地図のことで，一定量の放射線照射で切断されるゲノム DNA の長さが尺度となっている．正常細胞（私たちの場合は，ウシ正常細胞）の染色体を放射線照射により断片化した後，ハムスターなどの齧歯類由来の細胞（宿主細胞）と融合させ，100 種類程度の融合細胞から各々 DNA を回収してセットとした「RH パネル」を用いて，地図を作成する．RH パネルを構成する各 DNA には，断片化された正常細胞（ウシ）由来の染色体の一部が宿主細胞の染色体内に組み込まれて保持されているので，任意のマーカーを PCR 増幅することで，どの融合細胞 DNA がそのマーカーを含む染色体領域を保持していたか否かを知ることができる．ある2つのマーカーが近接していれば，各 RH パネルにおける PCR 増幅の有無のパターンは相似し，離れていれば相似しない．このような原理で，各マーカーの順番とマーカー間距離が算定できる．

RH 地図のマーカーは，連鎖地図のマーカーのように多型性を必要としないため，遺伝子配列からマーカーを設計してマッピングすることにより，遺伝子地区を作ることができる．一方で，RH マッピングは PCR 増幅の有無のみに依拠するため，エラーを除くことが難しい．そこで，RH 地図の信頼性を確保するためには，連鎖地図の情報を基本にしなければならない．つまり，連鎖地図上で染色体上の位置が特定されているマーカー，通常はマイクロサテライトマーカー，の並ぶ順番の情報を用いて，RH 地図のフレームワークを構築する作業を行う．照射放射線量が高いほど，染色体は細かく分断されて RH 地図の解像度は上がるものの，これらを正確につなぐためには，数多くのマーカーが必要になる．したがって，解像度の良い RH 地図を作るためには，高密度連鎖地図の作成が不可欠である．

RH 地図の有用性は，第一に，遺伝子地図を作ることができるということで，これによって，他の種，例えばヒトとのゲノム比較地図を作成することができ，比較地図を通じて，ヒトなどの豊富な遺伝子情報をはじめとするゲノム情報を利用できるようになることである．連鎖地図との統合によって，遺伝病などをマッピングした染色体領域が，ヒトのどの染色体領域に相当するのか，そこにはどのような候補遺伝子があるか，を一時に知ることができる．第二に，BAC 整列地図の作成やゲノム配列を染色体上に並べる際の足場として，重要である．

(3) BAC 整列地図

染色体 DNA を 150 - 200 kb 程度の大きさに切断して，大腸菌細胞内で複製できるように人工染色体に組み込んだものを BAC クローンといい，これを染色体上の順番で並べたものが BAC 整列地図である．BAC クローン DNA を特定の制限酵素で切断したパターン（フィンガープリント）同士を比較し，パターンの相同性に基づいてつなぎ合わせることによって，染色体上の順番に BAC クローンを並べることができる．このようにして並べた BAC クローンの一群をコンティグと呼ぶ．コンティグに含まれるマーカーを用いて，RH 地図と統合することにより，コンティグを染色体上に並べた BAC 整列地図ができる．

BAC 整列地図があるか否かは，ゲノム配列決定の質を左右する．ゲノム配列決定には，通常，全ゲノムを断片化した配列を全ゲノムの6倍長読んだものと，整列化した BAC クローンを全ゲノムの2倍長読んだものとを合わせて用いる．後者を加えることにより，配列間のギャップやつなぎ間違いの少ないゲノム配列が得られる．

3. 高密度連鎖地図の作成

ウシでは，1997 年に米国農務省肉畜研究センター（USDA MARC）のグループが，1,250 個の DNA マーカーを含む連鎖地図を作成した（Kappes ら 1997）．その後は，形質のマッピングにはこの程度の密度でも差し支えないことと，マーカーの開発には多大な労力と費用がかかることから，連鎖地図の更新はなされていなかった．しかし，疾病や経済形質をマッピングした後の原因遺伝子の同定を視野に入れた時，マッピングした領域ごとにマーカーを高密度に開発するのでは，大変に効率が悪い．そこで，全ゲノムを対象にランダムにマーカーを開発し，3,000 個のマイクロサテライトマーカーを含む連鎖地図を作ることを目標とし

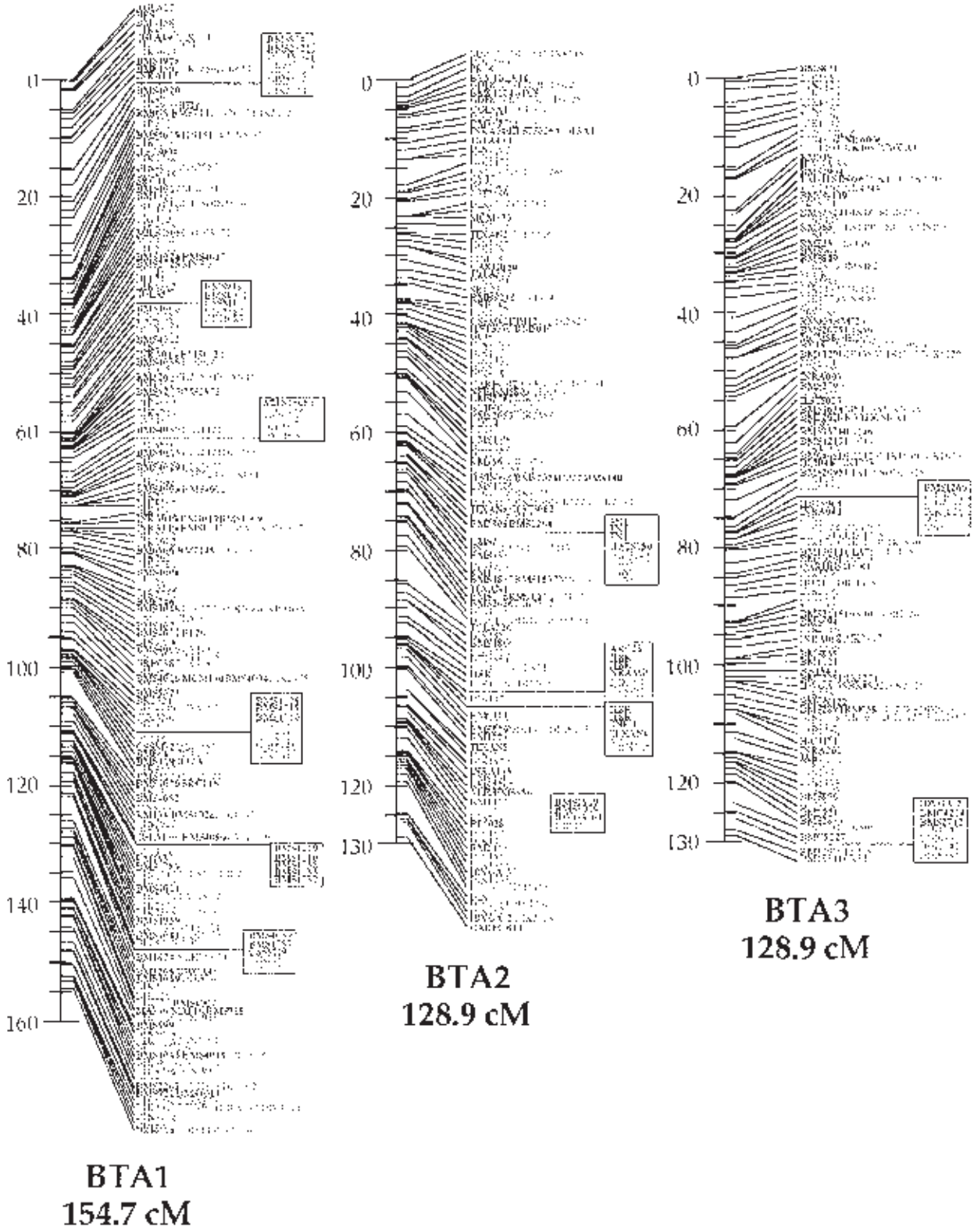

図 1.　Shirakawa-USDA 連鎖地図
第 1 番染色体から第 3 番染色体(BTA 1-3)までを示した．その他の染色体およびマーカー情報は，
http://www.marc.usda.gov/genome/cattle/cattle.html で閲覧できる。

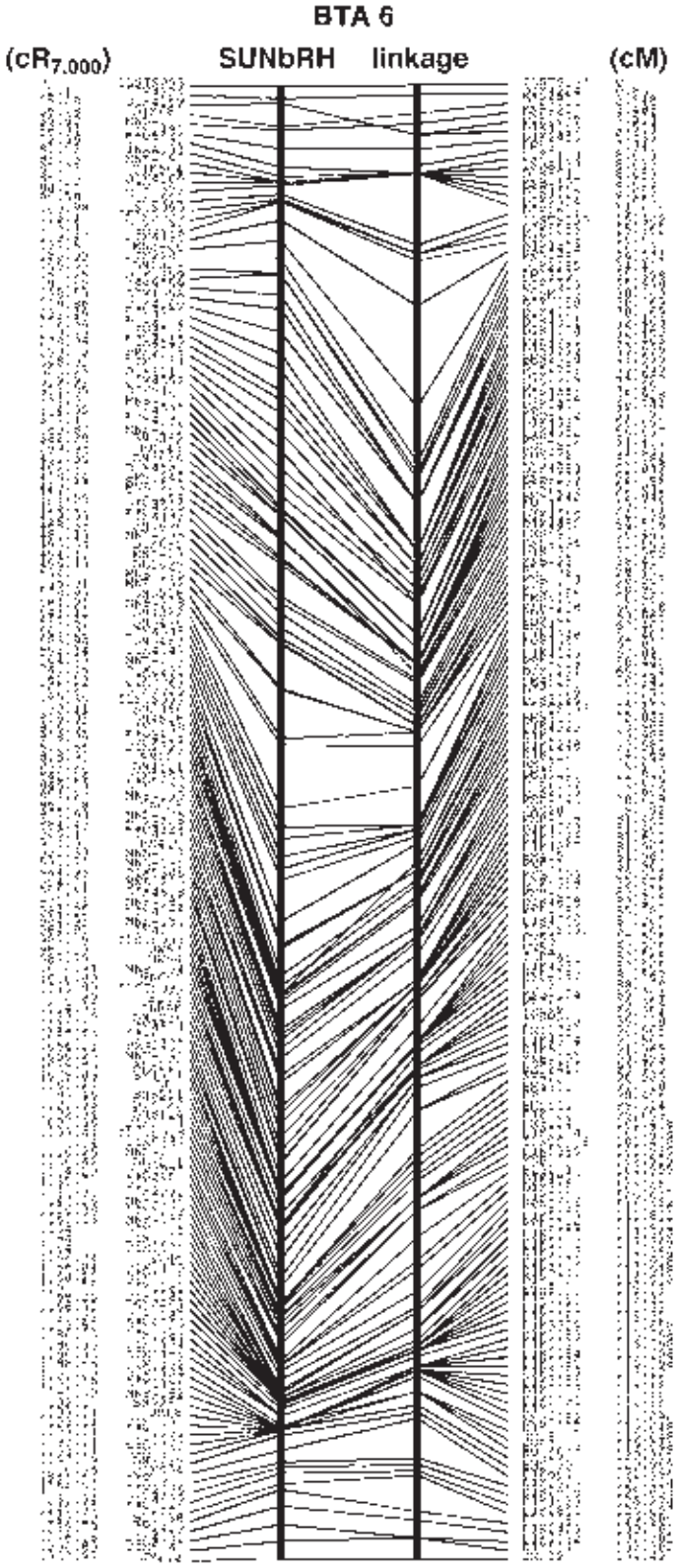

図2. SUNbRHフレームワーク地図と連鎖地図との対応
例として第6番染色体を示した。その他の染色体については、
http://www.animalgenome.org/cattle/maps/RHMap3/で閲覧できる。

で作成に取りかかった．そして，2,293個のマイクロサテライトをUSDA-MARCの連鎖地図に追加することによって，3,802個のマイクロサテライトマーカーを含む3,960個のマーカーから成る地図を作成し，Shirakawa-USDA連鎖地図として発表した（Ihara ら 2004）．

連鎖地図の解像度は，マッピングに用いる標準家系の規模に依存するので，USDA-MARCのウシ標準家系では，解像度の限界は0.8 cM（＝約800 kb）である．Shirakawa-USDA連鎖地図の3,960個のマーカーは，2,423ポジションの「点」であり，マーカー間隔は平均1.4 cMであった．連鎖地図の更新により，マーカー間隔が10 cMを超えるギャップは，X染色体の一箇所のみとなり，全ゲノムの51％はマーカー間隔2 cM以下で，また，91％は5 cM以下でカバーされた．この結果，疾病や経済形質のファインマッピングに十分なマーカー密度となり，また，RH地図の足場としても十分なものが作成できた．

4. 高密度RH地図とウシ-ヒトゲノム比較地図の作成

2000年に米国テキサスA&M大学のWomackらのグループが最初のウシ全ゲノムRH地図（5,000-rad放射線照射RHパネルによる）を報告した（Band ら 2000）．これは，319個のマイクロサテライトと768個の遺伝子から成り，ウシ-ヒトゲノム比較地図において105以上のシンテニーが観察されることがわかった．2002年には，英国のロスリン研究所やフランスの国立農業研究機構（INRA）などの共同で3,000-rad放射線照射RHパネルを用いた地図が報告された（Williams ら 2002）．これは，当時の連鎖地図にマップされていたマーカーから，できるだけ多くのマーカーを用いてフレームワークを構築しようとしたもので，1,148個のマイクロサテライトと90個の遺伝子から成っていた．前者のWomackらのRHパネルについては，イリノイ大のLewinらのグループが1,913個のマーカー（うち，667個をフレームワークマーカーとして使用）から成る第2世代の全ゲノムRH地図を作成し，2004年に報告した（Everts-van der Wind ら 2004）．しかしながら，これらのウシRH地図では，フレームワークに用いたマーカー数が充分とはいえず，信頼性および解像度に限界があると思われた．

私たちは，米国ミネソタ大（当時），ネバダ大（当時）のBeattie教授と共同で，7,000-rad放射線照射RHパネルを作成した．このRHパネル（SUNbRHパネル）を用いて，Shirakawa USDA連鎖地図上にマップされたマイクロサテライトマーカー3,216個を用いてフレームワークを作成した．図2をみると，連鎖地図と比較して，マーカー同士がよく分離しており，RH地図の解像度のよいことが一目でわかる．

このフレームワーク上に，2,377個の遺伝子またはESTをマップした．ESTとは，ゲノム上で発現している配列（遺伝子の一部分について塩基配列を決定したもののことをいう．ここで用いたほとんどのESTは，私たちが過去に開発したものである（Takasuga ら 2001；Itoh ら 2003）．

このようにして，計5,593マーカーから成るRH地図（SNUbRH地図）が完成した（Itoh ら 2005）．このうち1,716マーカーについて，ヒトゲノム上に相同配列を見いだすことができ，これらを用いてウシ-ヒトゲノム比較地図を作成した（図3）．少なくとも161個のシンテニーが観察され，特に4番，8番，10番，13番，19番などの染色体について，過去の報告と比べて，より複雑なシンテニーが観察された．私たちの比較地図で観察された全シンテニーは，ウシRH地図の79％，ヒトゲノムの72％をカバーしていた．また，RH地図1cRは，約114 kbに相当すると算定された．

5. BACフィンガープリント地図との統合

私たちの作成したRH地図が物理的な実体のある染色体地図を作成する上でどの程度有効かを，4番染色体を例に検証した（Itoh ら 2005）．前述のように，国際コンソシアムでは，BACフィンガープリント地図を作成中で，逐時，データをウェブサイトで公開している（http://www.hgsc.tamu.edu/lab/mapping/bovine/）．そこで，4番染色体のマイクロサテライトマーカーおよびESTについてBACクローンのスクリーニングを行い，ウェブサイトでこれらのクローンの属するBACコンティグを検索し，BACクローンの末端塩基配列とヒトゲノム塩基配列との相同性の情報も含めて，ウシ染色体上に並べてみた（図4）．その結果，私たちのRH地図は，BACコンティグをウシ染色体上に並べていくのに十分なマーカー数を持つことが確認された．また，私たちの比較地図は，BACクローンの末端塩基配列を相同性に基づいてヒトゲノム上に並べた結果と，よく一致しており，私たちの比較地図は十分な解像度をもつ，信頼性の高い地図であることが確認された．

6. ウシゲノム解読の現状

ウシゲノムプロジェクトは，Phase IとPhase IIから成り，Phase Iは，ウシゲノムの塩基配列決定から

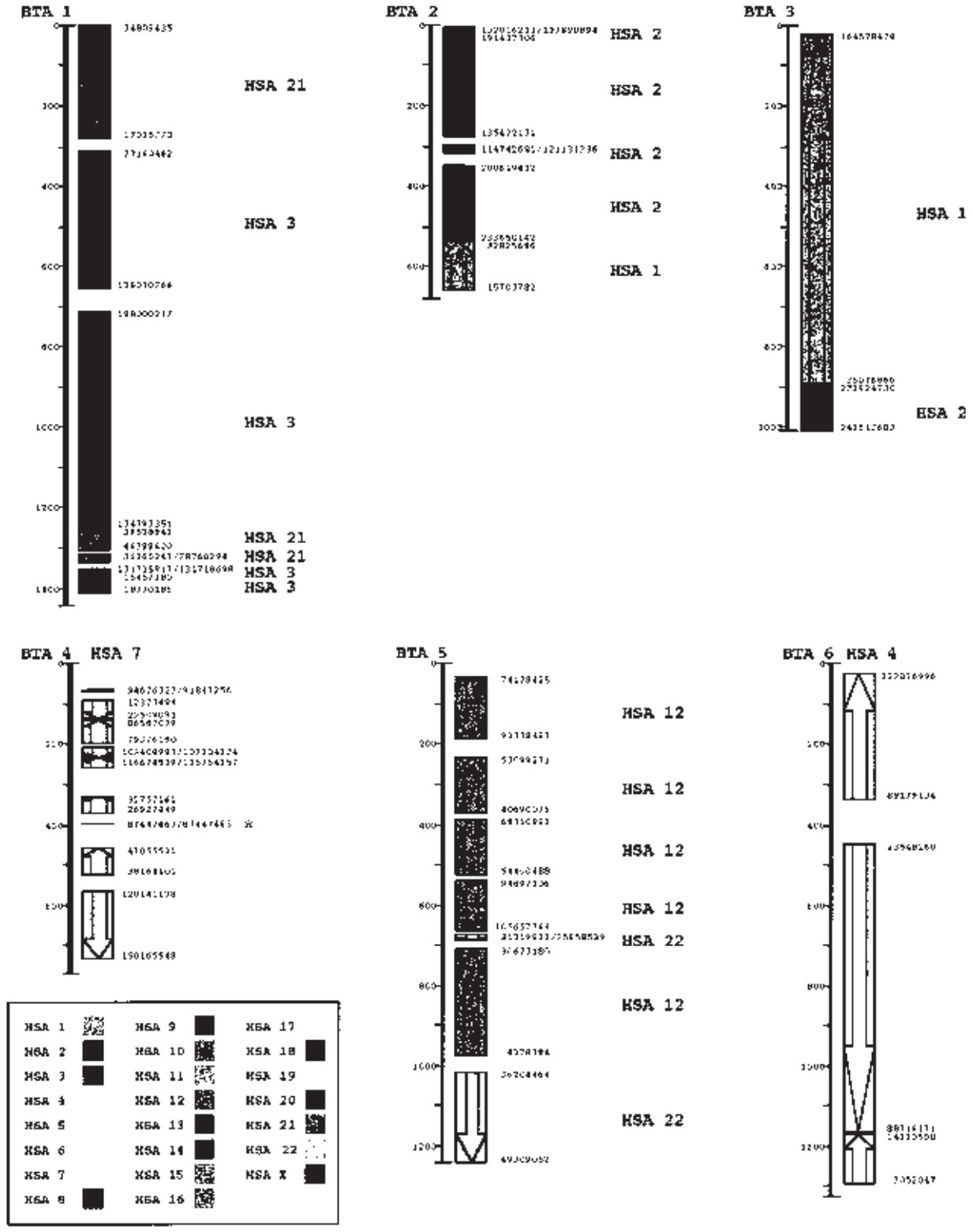

図3. ウシ－ヒトゲノム比較地図
第1番染色体から第6番染色体までを示した．ウシ染色体（黒太線、数字の単位はcR）上に、ヒト染色体との相同領域（シンテニー）を、...で示した．...の右側の数字は、ヒト染色体の塩基配列番号（単位は、bp）を示し、_内の矢印は、シンテニーの向きを示す．その他の染色体については、Hoh らの文献(2005)を参照されたい．また、これらの詳細は、http://www.animalgenome.org/cattle/maps/RHMap3/で閲覧できる．

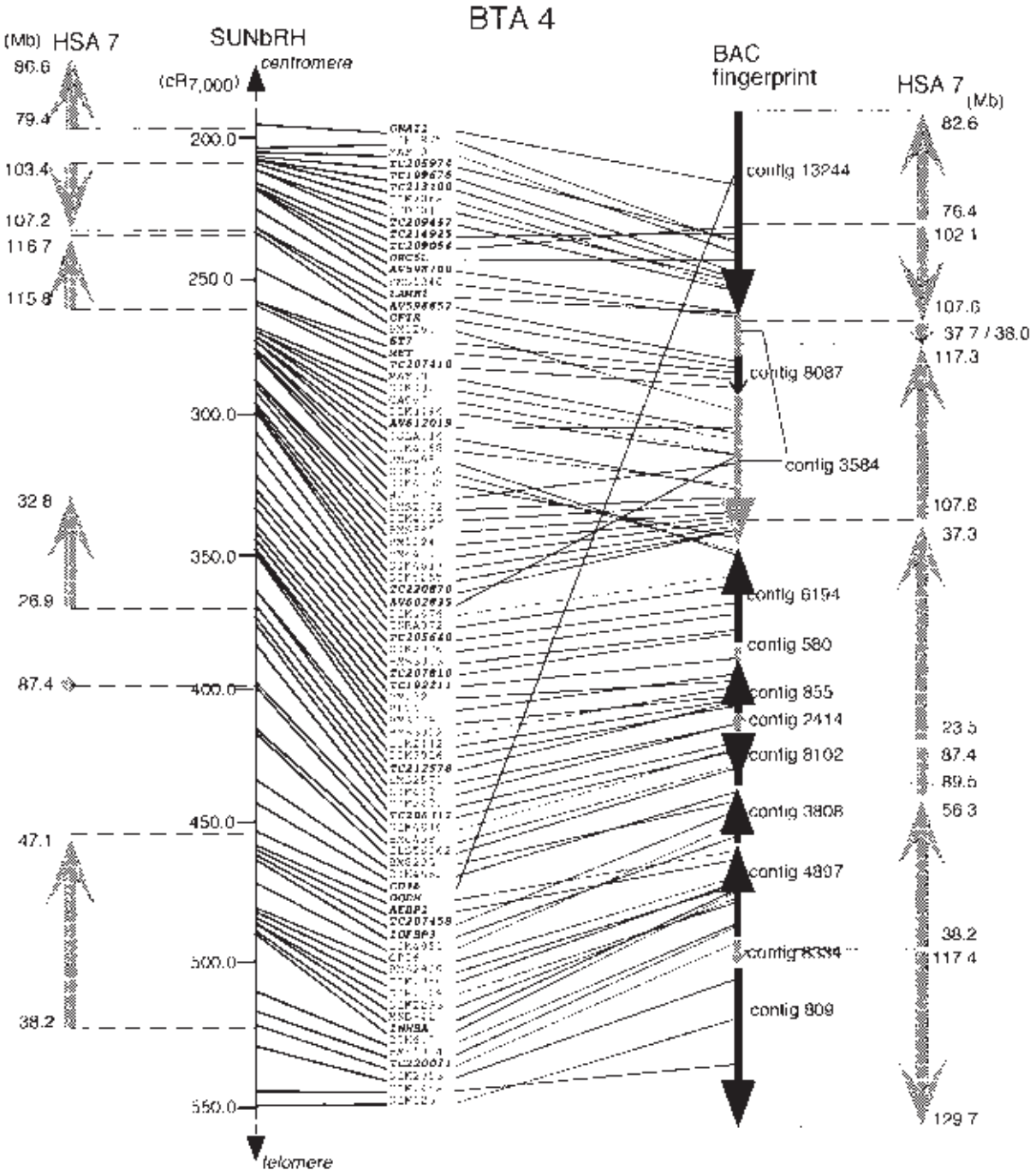

図4. SUNbRH 地図を用いた BAC コンティグの整列化
ウシ第4番染色体上の霜降りの肉質に関与する領域について（Mizoshita ら 2004）、SUNbRH 地図と BAC フィンガープリント地図の情報を用いて、BAC コンティグの整列化を行った。SUNbRH 地図上のマーカーでスクリーニングした BAC クローンの BAC コンティグ内での位置を線で結んでいる。左端は SUNbRH 地図の、右端はコンティグに含まれる BAC クローンの末端塩基配列を用いた場合のヒトゲノムとの比較地図を示す。BAC コンティグを整列化することによって、ヒトゲノムとのシンテニーが、より詳細に明らかになった。また、この時点で用いた BAC コンティグの情報には誤りがあることも明らかになったが、(contig8087 は contig3584 の間に入る)、その後の改訂で修正されている。

Annotaion までを、Phase II は、SNP 検索と各品種の HapMap の作成を目的としている。

Phase I については、先に触れたように、すでに、ゲノムの 6 倍長の WGS 配列が公開されているが、現在、ゲノムの 2 倍長の BAC skim (フィンガープリントで整列化した BAC クローンを、単一、もしくは、プールして、配列を読んだもの) が進行中である (http://www.hgsc.bcm.tmc.edu/projects/bovine/)。現在用いられているゲノム塩基配列決定の手法は、WGS と BAC skim を組み合わせたもので、BAC skim により、配列間のギャップが埋まり、また、リピート配列によるつなぎ間違いが改善されることが期待される。実際、現在公開されている 6 x WGS 配列は、92 %の EST と 96 %の BAC 末端配列を含んでいて、遺伝子の Annotation にはほぼ十分であると考えられているが、WGS 配列の半分は 434 kb 以下の長さで、特に、遺伝子のプロモーター領域に相当する CpG island 部分はギャップになっていることが多い。

WGS 配列と BAC skim 配列は、アセンブルされて scaffold 配列となった後、scaffold 配列が含むマーカーによって、地図を通してウシ染色体上に配列される。ウシゲノムプロジェクトにおいて、高密度地図の必要性が認識されるのに伴い、前出の 5,000-rad と 3,000-rad の RH 地図の完成も図られている。Lewin らのグループは、2005 年 12 月に、3,484 個のマーカーから成る第 3 世代の RH 地図を報告した (Everts-van der Wind ら 2005)。これは、2,516 個の BAC 末端マーカーを含んでおり、BAC フィンガープリント地図との統合を図ったものである。また、カナダの Moore らのグループは、3,000-rad の RH 地図に、多数の SNP をマッピングし、WGS 配列との統合を図っている (McKay ら 2006)。また、米国ミズーリ大の Taylor らは、14 世代から成るアンガス種の家系を用いて、多数の SNP をマッピングした連鎖地図を作成している。国際コンソシアムでは、現在これらすべてを含んだ統合地図 (Composite map) を作成中である。この統合地図を用いて、scaffold 配列がウシゲノム上にアセンブルされる見込みである。

一方、Phase II は、SNP 検索と HapMap の作成を目的としている。すでに、WGS にヘレフォード種を使用、と、その他 6 品種 (アンガス、ブラーマン、ホルスタイン、シャージー、リムジン、ノルウェーレッド) のゲノムシークエンシングにより、一万個以上の SNP が見出されている。今後、これらの SNP が各品種でタイピングされ、ハプロタイプブロック等が明らかにされていく予定である。

7. おわりに

動物遺伝研究所が取り組んできたゲノム解析用ツールの開発は、時代の流れに沿って、ウシゲノムプロジェクトへ受け継がれた。連鎖地図の高密度化を行い、その結果を利用して構築したフレームワークに基づいた RH 地図の作成は、ウシゲノム配列決定へのきわめて重要な貢献であったと位置付けられるだろう。Phase I で完成度の高いウシゲノム配列が得られることか、Phase II のウシ HapMap の作成にも重要である。SNP の正しい位置が得られなければ、ハプロタイプブロックの解析などできないからである。Phase II が完了する数年後には、ウシ品種間の差か、SNP レベルで明らかにされ、ヒトと同じように、ウシにおいても SNP アレイを用いて全ゲノムを対象にした相関解析を行える基盤ができるだろう。その時には、ゲノム解析に関して、皆が同じスタートラインに立つことになる。今、その先を見通した戦略が必要とされている。

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