FINDING GENES THAT CONTROL BODY SIZE AND COMPOSITION: THE MOUSE MODEL

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Story in Brief

Future genetic improvement of livestock may be enhanced by new technologies such as genetic engineering (gene transfer) or use of DNA information to increase accuracy of selection (marker assisted selection). However, before these technologies can be useful, we must first be able to identity which genes are the most important in regulating traits of potential economic importance. To date, little such information is available, and research designed to identify these genes in livestock species is both extremely expensive and time consuming. Therefore, the objective of the present study was to identify genes that play significant roles in the control and regulation of components of growth and body composition in mice, a model species. To accomplish this, a large backcross population was produced by crossing two unique and different inbred lines, M16i and CAST. M16i is the result of long-term selection for postweaning (3-6 wk) weight gain followed by 15 generations of full-sib mating, and is characterized by large body size and obesity. CAST is an inbred line of wild origin, and is characterized by small body size and a lean carcass. Growth and body composition phenotypes were collected, and genotypes were determined for random DNA markers evenly dispersed throughout the genome. Several markers which may be linked to genes with significant effects were identified for both mature body weight and percent body fat. The average effect of these markers was in the range of 1 to 2% of the phenotypic variation. One marker was identified which may be linked to a gene with much larger effects on body size and composition.

(Key Words: Genes, DNA, Growth, Body Size, Body Composition, Mice.)

Introduction

Identification of molecular markers that are linked to important genes (quantitative trait loci; QTL) is a prerequisite for marker assisted selection in animals, as well as for determining the identity of the QTL and elucidating the genetic control of economically relevant traits. A thorough screening of the

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genomes of livestock species for markers linked to QTL has been difficult due to the lack of highly saturated marker maps and large, segregating resource families. The time and expense involved in producing these populations is also an important factor in limiting the capabilities of researchers attempting to find QTL, especially in cattle. In contrast, the mouse is a very useful model species for detecting QTL. Advantages include extreme phenotypic divergence due to the availability of a variety of selection lines, the ability to have fully informative DNA markers due to inbreeding and a saturated marker map, and a high power of QTL detection due to the use of very large population sizes and controlled environment.

Highly polymorphic markers are frequently obtained when the polymerase chain reaction (PCR) is used to amplify repetitive sequences of base pairs of DNA. This is the basis for one of the most useful classes of genetic markers, termed microsatellites. Microsatellites are tandem arrays of short (1 to 4, but usually 2, bp) repeats that are subject to great variation in number of repeat units. Because of their ubiquity, frequency of polymorphism and ease of detection, microsatellites have rapidly become the marker of choice for genome studies in a variety of species, including cattle (Bishop et al., 1994), pigs (Rohrer et al., 1994) and mice (Copeland et al., 1993). Well over 2,000 microsatellite markers have already been developed and mapped for use in the mouse (Copeland et al., 1993), and it was the objective of this research to combine this resource with the availability of unique selection lines in an attempt to map QTL that explain variation in traits related to growth and composition.

Materials and Methods

Origin and Husbandry of Mice: Mice used in these studies were from the M16i selection line (based on long-term, within full-sib family selection for high postweaning gain from 3 to 6 weeks (Hanrahan et al., 1973) followed by 15 generations of inbreeding by full-sib mating) and from the inbred line of wild origin, CAST/Ei (CAST; a strain of *M. m. castaneus*). M16i mice are characterized by very large body size and obesity, while CAST mice are characterized by small body size and lean carcasses. All mice were reared in the Mouse Genetics Laboratory at North Carolina State University at a temperature of 21°C, 55% relative humidity and a light:dark cycle of 12 h:12 h beginning at 0700. Mice were allowed *ad libitum* access to Purina Laboratory Chow from weaning until mating and Purina Mouse Chow from mating until weaning.

Production of a Segregating Backcross Population: A large backcross population was created from an initial cross of M16i and CAST. Cast males were mated to M16i females, and 7 F1 males were backcrossed to M16i females, resulting in 54 litters with a total of 424 mice reaching adult age (12 wk). Body weights were collected on backcross individuals at 3, 6, 9 and 12

wk. At 12 wk, all mice were killed and evaluated for fat pad (gonadal and subcutaneous) and organ (liver, kidney, spleen, heart, testis) weights.

Marker Detection: DNA was extracted from tail clips with a phenol/chloroform - isopropanol precipitation method. A whole-genome PCR-based DNA marker screening strategy was developed, utilizing three relatively even spaced polymorphic markers for each of the 19 mouse autosomes (a backcross does not enable screening of sex chromosomes). Microsatellite markers were selected from the well saturated mouse marker map (Dietrich et al., 1992; Copeland et al., 1993) and tested for polymorphisms between M16i and CAST parental DNA and ease of detection using simple agarose gel and ethidium bromide staining methods.

All primers for PCR (MapPairs) were purchased from Research Genetics (Huntsville, AL). PCR reactions were performed in 15 µl volumes, overlaid with 50 µl light mineral oil (Sigma), in U-bottom PVC 96-well microtiter plates (Falcon 3911) using the MJ PTC-100 Programmable Thermal Controller. Each reaction consisted of 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X, 200 µM each dNTP (Boehringer Mannheim), 1.5 mM MgCl2, 200 nM of each primer of a MapPairs set, 0.375 U *Taq* Polymerase (Promega), and 30 ng genomic DNA. PCR cycle conditions included initial denaturation at 94°C for 3 min prior to cycling followed by 40 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 2 min and extension at 72°C for 2 min. An additional final extension step was carried at 72°C for 7 min to insure full extension of all products.

PCR products were analyzed in 4% sieving agarose gels (2% NuSieve low melting point agarose, FMC BioProducts; 2% Ultrapure Biotechnology Grade high melting point agarose, BioRad). Gels were run for 75 min at 80 V, followed by staining in ethidium bromide for 30 min and destaining in ddH2O for 30 min. PCR products were visualized and photodocumented with UV illumination.

Selective Genotyping: Two traits were selected as candidates for initial QTL detection: 12 wk body weight, and gonadal fat pad weight as a percentage of 12 wk body weight (indicator of overall body fat). Selective genotyping (Lander and Botstein, 1989) was employed to identify those markers which have an increased likelihood of being linked to a QTL. For each trait, the highest and lowest ~10% (20 mice) of each sex were selected for genotyping.

Statistical Analysis: For each marker screened in the backcross population, there was an expectation of 50% M16i/M16i homozygous genotypes and 50% M16i/CAST heterozygous genotypes. When a deviation was found to be significant by Chi-Square analysis for either 12 week body weight or gonadal fat percentage, the remainder of the population was genotyped for that marker. Whole population data were analyzed with least-squares procedures to quantify

the effect of the individual marker on the two traits of interest. The model included effects of litter, sex, genotype and the sex x genotype interaction.

Results

Descriptive Statistics of the Backcross Population: Descriptive statistics for the two primary traits (12 wk body weight and gonadal fat pad percentage) in this backcross population are presented in Table 1. A very wide range of phenotypes around the mean was created.

Means of the high and low tails of the population are also presented in Table 1. By utilizing widely divergent parental lines and a large informative segregating population, wide phenotypic variation was created for each trait, increasing the probability of QTL detection. This provides an excellent base for preliminary detection of QTL which explain significant proportions of these differences.

Selective Genotyping: Genotyping of the 10% phenotypically highest and lowest mice for each trait was successful in that several markers exhibited significant deviations from expected segregation ratios within the tails. For 12 week body weight, 13 of the 57 markers had a significant deviation ($P<.05$) in at least one of the two tails, while six of these deviated in both phenotypic tails. For fat percent, 7 markers deviated from expectations in at least one tail, with four exhibiting deviations in both tails. Selective genotyping indicated that three markers (on chromosomes 2, 15 and 18) may have strong pleiotropic effects, because they deviated from expected segregation ratios in both tails of both traits.

Marker Effects on Phenotype: Markers which appeared promising based on results of selective genotyping were typed for the remainder of the population. Results are summarized in Table 2. On average, phenotypic variation accounted for by significant markers was in the range of 1% to 2%. The exception was marker D2MIT49, which resides on the distal third of chromosome 2, which may be linked to a pleiotropic QTL with particularly strong effects on both body weight and fat. Substitution of the CAST allele with the M16i allele at this marker led to a 9.8% increase in 12 wk body weight and a 38% increase in body fat.

Discussion

Results of the present study are preliminary in that the entire backcross population has yet to be genotyped for all markers, and statistical analyses such as those utilizing a combination of multiple regression and interval mapping (Zeng, 1994) have not been employed. Once those goals are met, a clearer picture of chromosomal location of QTL for body weight and body fat will be achieved. At that time, chromosomal walking will be conducted to perform

Table 1. Descriptive statistics of the M16i/CAST x M16i backcross population (n=424) for two example traits (adult (12 wk) body weight and epididymal fat pad weight as a percentage of body weight).

Trait	Sex	Mean	SD	CV	Range	10% Low[a]	10% High[a]
12 wk wt, g	M	45.27	5.86	12.96	18.1 - 67.1	35.95	54.89
	F	35.70	4.72	13.22	21.0 - 50.8	27.64	43.98
Fat %	M	1.17	.47	40.37	.17 - 2.69	.48	2.14
	F	.46	.39	84.69	.01 - 2.43	.10	1.20

[a] Represents the mean of the 10% highest and lowest mice within each trait separately.

Table 2. Preliminary findings[a] on QTL for growth and fat in the M16i x CAST backcross population.

		12 week body weight (g ± s.e.)			Epididymal fat pad (% ± s.e.)		
Chrom	Marker[b]	Mean M/M[c]	Mean M/C[c]	RSQ[d]	Mean M/M[c]	Mean M/C[c]	RSQ[d]
2	D2MIT49	42.6 ± .3	38.6 ± .3***	6.5%	.97 ± .03	.66 ± .03***	6.5%
6	D6MIT50	41.3 ± .3	39.8 ± .3**	1.0%	.84 ± .03	.78 ± .03	----
11	D11MIT5	41.5 ± .3	39.6 ± .3***	1.6%	.81 ± .03	.81 ± .03	----
15	D15MIT34	41.6 ± .3	39.7 ± .3***	1.6%	.88 ± .03	.75 ± .03**	1.3%
18	D18NDS1	41.4 ± .4	39.4 ± .4***	1.4%	.93 ± .04	.78 ± .04**	1.5%
19	D19MIT11	40.8 ± .8	40.3 ± .8	----	.89 ± .04	.71 ± .04**	1.8%

[a] Data represent only those markers where selective genotyping indicated significant deviations in both tails for either weight or fat traits.

[b] Marker designations are from Dietrich et al. (1992).

[c] M/M = M16i/M16i homozygous genotype; M/C = M16i/CAST heterozygous genotype.

[d] RSQ = partial R-square value indicating relative proportion of total variation explained by the marker.

* P<.05, **P<.01, ***P<.001 (comparing genotypes for each marker within each trait class). N=400 to 420, except for D19MIT11 where only half of the population has been genotyped.

fine scale mapping in an attempt to identify the specific gene that represents the detected QTL. Since the mouse map is saturated with microsatellites, many markers exist in close proximity to the ones used in the initial screening process. These markers will be screened until a marker is found that is as close as possible to the putative QTL. Many genes which may influence quantitative traits of economic importance have already been mapped (Copeland et al., 1993). Genes in close proximity to the final QTL marker location will be considered as candidate QTL. Candidates for the QTL that are linked to the markers listed in Table 2 include: growth hormone releasing factor for D2MIT49, fatty acid binding protein or transforming growth factor alpha for D6MIT50, and growth hormone or IGF-1 binding protein for D11MIT5.

The average phenotypic effect, in terms of variation explained, of most of the significant markers identified thus far in this study is in the range of 1% to 2%. Although preliminary, this would agree well with the general model which states that most quantitative traits are controlled by many genes, each with small effect. However, one of the significant markers identified on chromosome 2 appears to be linked to a QTL with more of a major effect on both body weight and body fat. Many markers that may be linked to QTL with pleiotropic effects were identified, which is expected based on the high genetic correlation that exists between body weight and body fat in mice. Additionally, there is evidence of significant sex x marker interaction effects on both growth and body fat in these preliminary results. This too would fit expectations, since it is likely that the phenotypic effect of many genes may be influenced by sex.

One of the deficiencies of the backcross design utilized in this study is that it only enables estimation of markers linked to QTL with additive effects. Studies are presently in progress utilizing the more informative F2 design. Additionally, other long-term selection lines are being incorporated into crosses, expanding the list of traits for which QTL will be identifed to include components of reproduction and maternal performance.

Gene mapping investigations in the mouse should help to answer questions regarding how many QTL exist for economically relevant traits and what their relative effects are. Moreover, QTL identified in the mouse may have a high probability of being QTL in livestock species as well. There is currently rapid development of comparative maps between humans, mice, cattle, sheep and pigs. The mapping of QTL in mice may thereby immediately expose candidate chromosomal segments for identification of homologous QTL in livestock species. We plan to utilize the mouse model not only to identify and map QTL that may also exist in livestock species, but also to examine questions related to design and analysis of QTL identification studies, in order to help optimize such studies when conditions are not as ideal.

Marker assisted selection, or the use of information on an animal's DNA to increase the accuracy of identification of animals with the best breeding values, should prove useful in enhancing genetic improvement of livestock in the future. However, much information needs to be obtained on the

chromosomal location and identity of QTL before marker assisted selection can become a reality. Thorough and comprehensive screenings of genomes of livestock species will be required, as opposed to the candidate gene approach that is currently being used most frequently, whereby individual genes are analyzed one by one as potential QTL. Studies with model species such as the mouse should prove very beneficial in facilitating future identification of QTL in livestock.

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Acknowledgments

This research was supported in part by a grant from the Oklahoma Center for the Advancement of Science and Technology (OCAST) - Health Research Program. The technical assistance of Grady Beck and Dana Ross in the laboratory is gratefully acknowledged.