

# GROWTH HORMONE GENE POLYMORPHISM IN LINES OF MICE SELECTED FOR GROWTH ON TWO DIETS

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## SUMMARY

Polymorphism in the growth hormone gene was detected with the restriction enzyme *Hind*III in lines of mice selected for high and low growth on normal and reduced protein diets. The frequency of an allele identified by a *Hind*III fragment of 4.5 kbp was highest on the normal protein diet, where the allele was nearly fixed in the line with the highest growth. An association of this variant with high growth on the normal protein diet may explain the observation.

## INTRODUCTION

Animal breeding may be improved by combining traditional results on performance with molecular genetic information on quantitative trait loci (QTLs) in a selection index. A QTL may be identified from genetical and physiological knowledge, and the frequency of variants in a candidate gene is expected to change as a result of selection for the quantitative trait.

Genes in the somatotropic axis are likely QTLs as their products are known to be important in the regulation of growth processes, and recent experiments have shown differences between frequencies of growth hormone (GH) alleles in lines of mice selected for high and low body weight (Winkelman and Hodgetts, 1992) and in lines of cattle selected for high and low milk fat production (Høj et al., 1993).

In the present investigation the frequency of alleles in the GH gene and the association of genotypes with growth were studied in lines of mice selected for high and low growth and in control lines on two protein diets.

## MATERIALS AND METHODS

**Animals.** The establishment of the base population (generation 0) for the experiment is described by Nielsen and Andersen (1987). In generation 0 the offspring groups were divided into three blocks (1, 2, 3). From each block, parents were obtained for the establishment of a selection line (S) and a control line (C) on each protein level (N, R) by randomly taking two male and two female offspring from each offspring group and placing sibs of opposite sex on different protein levels. From the sibs on the same protein level, one was used in the selection line and the other in the control line.

The final selection lines were established by selecting in generation 1, within each litter, the male and female with the largest growth from 3 to 9 weeks of age for the high line (H) and the male and female with the smallest gain for the low line (L). In the control lines (C) a male and female were chosen at random within each litter. Each line consisted of 8 pair-matings per generation.

In the selection experiment, litters were standardized at birth to eight young, four of each sex. Litters were weaned at 3 weeks of age, the males and females were separated, and a maximum of four mice were kept per cage. Selection was performed within litters. Mice used for the present experiment were from generation 14, where the lines had been selected for 11 generations. No selection was performed in generation 7 and 9. One male mouse from each family in each line was used for the investigation.

**Diets.** The normal protein diet (N) contained 19.3% crude protein, while the protein content was limited to 5.1% in the reduced protein diet. The reduced protein diet caused a reduction in growth as described by Nielsen and Andersen (1982).

**GH gene polymorphism.** Genetic variation was revealed as restriction fragment length polymorphism (RFLP) using common molecular biological methods among these Southern Blotting.

**Statistical analysis.** Statistical analysis of gene frequencies was performed using a Chi-Square test.

Growth from 3 to 6 weeks of age was analysed using the following model, where  $y_{ijklm}$  denotes growth from 3 to 6 weeks of age on diet  $i$  ( $i = N, R$ ), selection direction  $j$  ( $j = H, C, L$ ), block  $k$  ( $k = 1, 2, 3$ ), GH genotype  $l$  ( $l = H45/H45, H45/H42, H42/H42$ ) and individual  $m$  ( $m = 1, 2 \dots 8$ ):

$$y_{ijklm} = m + diet_i + sel_j + block_k + ghgeno_l + diysel_{ij} + selblock_{jk} + e_{ijklm}$$

- $m$  = the general mean;  
 $diet_i$  = the fixed effect of  $i$ th diet;  
 $sel_j$  = the fixed effect of  $j$ th selection direction;  
 $block_k$  = the random effect of  $k$ th block;  
 $ghgeno_l$  = the fixed effect of  $l$ th GH genotype;  
 $diysel_{ij}$  = the fixed effect of the interaction between the  $i$ th diet and the  $j$ th selection direction;  
 $selblock_{jk}$  = the random effect of the interaction between the  $j$ th selection direction and the  $k$ th block; and  
 $e_{ijklm}$  = is the random error term.

The data were analysed using the GLM Procedure (SAS 1989).

## RESULTS

Polymorphism in the GH gene was detected with the restriction enzyme *HindIII*. Digestion with *HindIII* revealed animals with one fragment of 4.2 kbp (H42/H42), animals with one fragment of 4.5 kbp (H45/H45) and animals with both fragments (H42/H45). The two fragments are assumed to be allelic. The number of genotypes and the mean growth from 3 to 6 weeks of age in the lines are given in Table 1.

**Table 1.** Number of GH genotypes revealed after digestion with *HindIII* and mean growth from 3 to 6 weeks of age in male mice from the lines on the two protein diets (N, R), and the three blocks (1, 2, 3) selected for high or low growth or as control (H, C, L).

LINE	NH1	NH2	NH3	NC1	NC2	NC3	NL1	NL2	NL3
GENOTYPE									
H42/H42	6			8	7	1	6	2	
H42/H45	1	7			7	1	4	3	
H45/H45	6								
Growth (g)	10.6	15.2	8.1	3.8	8.1	12.4	6.7	8.1	8.5
Std. dev.	3.1	1.5	5.0	2.0	2.4	3.0	3.0	3.0	4.8

LINE	RH1	RH2	RH3	RC1	RC2	RC3	RL1	RL2	RL3
GENOTYPE									
H42/H42	6	7	7	6	5	2	4	7	6
H42/H45			1		1	1	1		
H45/H45									
Growth (g)	12.5	11.0	13.0	7.3	8.7	8.9	5.4	5.7	5.0
Std. dev.	2.8	2.8	2.3	1.4	0.9	2.1	1.7	1.6	1.1

Ignoring blocks the frequency of the H45 allele in male mice on the two diets and the three directions of selection was 0.50, 0.15, 0.25, 0.02, 0.07, and 0.03 in the lines NH, NC, NL, RH, RC, and RL, respectively. These frequencies were significantly different ( $P < 0.001$ ). The frequency of the H45 allele was considerably higher in mice kept on the normal protein diet, and animals homozygous for this allele were only found in the NH2 line.

The analysis of growth from 3 to 6 weeks of age revealed that a significant part of the variance was

attributable to GH genotypes ( $P=0.003$ ). The estimate of the effect on growth was largest for the homozygote H45/H45, while the estimates were similar for the two other genotypes. Further, a significant effect of the interaction between diet and direction of selection was found ( $P=0.003$ ), and also the interaction between selection direction and block contributed significantly to the variance ( $P=0.0006$ ).

#### DISCUSSION

In a study of growth-selected mice and an unselected foundation population, Salmon et al. (1988) found similarly a polymorphism with the restriction enzyme *HindIII* in the GH gene. The fragment sizes obtained were 4.5 kbp and 8.0 kbp. Only the 4.5 kbp fragment was similar to the investigation made by Salmon et al. and the experiment reported here. The presence of an allele unique to each of the experiments is probably due to the substantial difference in lines of mice used for establishment of the base population for the investigations.

Salmon et al. (1988) found the haplotype with the 4.5 kbp *HindIII* fragment to be fixed in growth selected lines and later Winkelman and Hodgetts (1992) found that this haplotype increased in lines selected for high weight and decreased in lines selected for low weight. The result of the present experiment was not as clear, but on the normal protein diet the highest frequency of the H45 allele was found, when selection was performed for high growth and in the NH2 line the allele was nearly fixed. Also the highest growth was found in this line. Contrary to this, the NH1 line was fixed for the H42 allele and in the NH3 line all mice were heterozygotes. Knowledge of the gene frequencies in the base population is essential for the interpretation of these results. Also the possibility of a confounding of genotype and line effects should be considered.

Winkelman et al. (1990) suggested that the effect of the GH haplotype with the 4.5 kbp *HindIII* fragment may be dependent on the genetic background. In an earlier investigation of the lines in the present experiment, a low genetic correlation was found between growth on the two protein levels, which indicates that growth on the two diets has a rather different genetic basis (Nielsen and Andersen, 1987). A difference in the effect of the H45 allele on the two diets may explain the considerably lower frequency of the H45 allele on the reduced protein diet than on the normal protein diet. Sampling in the base population and genetic drift may explain it as well.

Notable is that all mice from block 3 on the normal protein diet are heterozygotes, however with one exception. A balanced lethal system introduced into block 3 can explain this observation. Most of the founder mice for this experiment were from inbred lines, but also one mouse from a wild Danish population was used. This mouse could have been the carrier of the lethals.

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