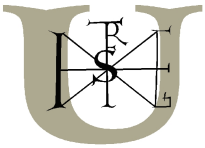

Szent István University

INVESTIGATION OF HYPERMUSCULARITY MODIFIERS ON COMPACT MOUSE CHROMOSOME X

Thesis of Ph.D. dissertation

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Gödöllő

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1. BACKGROUND AND OBJECTIVES

Several examples verify genetic heterogeneity of the hypermuscled (*i.e. double muscled or DM*) phenotype of cattle breeds. Initially, genetic determinability was mainly attributed to the *mh* (muscular hypertrophy) gene being a major gene, and was later described as myostatin (McPherron et al., 1997). This gene plays a vital role in determining the rate of muscle growth. In the cattle species several of its mutations are known, but six - that result in the inactive form of the coded protein - stand out (Karim et al., 2000). These in the homozygous form and as combined with each other create the myostatin „null genotype", thus the double-muscled character in several beef cattle (such natural myostatin mutations inhibiting protein functions had already been described in several species: mouse, human, sheep, dog and pig). In the Belgian Blue cattle breed double muscling is breeding aim and main breed characteristic. The study by Charlier et al. (1995) reveals that during breeding the muscling of the breed became much more expressed. This means that - after *mh* gene fixation - during selection aimed at muscling most probably further genes (modifying factors, modifier genes) became involved. The genetic background of hypermuscularity is therefore much more complex than originally thought. The following research results further support the complex genetic view of the double-muscled phenotype, showing the extremes, the two extreme cases of the genotype-phenotype relationship.

- **There is** inactivating myostatin **mutation**, but **no** double-muscled **phenotype**: The characteristic inactivating myostatin mutation of the Belgian Blue cattle can be found in the South Devon cattle breed as well. However, due to the deliberate breeding work against the double-muscled phenotype and its associated delivery problems, individuals homozygous to the mutation are well muscled, but did not show definite double-muscled phenotype. Smith et al. (2000) explained this phenomenon with the contribution of additional loci.

- **There is no** inactivating myostatin **mutation**, but **there is** double-muscled **phenotype**: In some beef cattle varieties (limousine and blonde d'aquitaine) the animals do not carry inactivating myostatin mutation despite of the double-muscled phenotype. This also raises the possibility that other factors - in addition to myostatin - also influence muscular hypertrophy (Grobet et al., 1998).

Therefore the mutant myostatin is a key determinant of excessive muscle mass development in several species, however, within a specific species or even within a breed several effective myostatin mutations occur, the effect of which can be further modified by further genes. Several of the effective cattle myostatin mutations are known by now already, however, the factors actually modifying them are unknown. These modifier genes are highly significant also because by learning

more about them a more detailed picture can be drawn for the process of muscle development, its control, which thus - knowing the conserved function of myostatin - may point beyond its utilization in animal husbandry. Their genetic mapping would be a time consuming and difficult task due to the interval of cattle species generations and due to the nature of modifying factors having only a small effect in themselves. Comparative mapping and the use of special mapping populations of laboratory model species may, however, bring breakthrough in these cases as well. The Genome Mapping in Animals research group of Dr László Varga conducted scientific research in this area (Agricultural Biotechnology Center). As part of their work they investigated and mapped the genetic background of the so-called Compact mouse, which is a natural hypermuscled mutant. Based on their earlier work they determined the myostatin gene sequence of the Compact mouse and thus discovered its mutation. Afterwards, they were the first to map the myostatin gene on mouse chromosome 1; up until then its position was unknown. This 12 base pair myostatin deletion ($Mstn^{Cmpt\text{-}dl1Abc}$) is a non inactivating mutation (affects the propeptide region of the protein). Thus $Mstn^{Cmpt\text{-}dl1Abc}$ is a necessary but not sufficient criterion of the development of hypermuscularity. During genetic analyses the research group established that the Compact phenotype is formed by several modifier loci of different strength (located on chromosome 1, 3, 5, 7, 11, 16 and X) together with the main gene. From among them it was the locus detected on the X chromosome that showed the most powerful, and at the same time most extensive modifier effect (Varga et al. 1997, Szabó et al. 1998, Varga et al. 2003a, Varga et al. 2005).

Objectives of my work:

- Sequence and expression level investigation of the androgen receptor gene (Ar) located in the most powerful section of the X chromosomal modifier region in the Compact mouse. This gene is a candidate myostatin modifier not only due to its position but also based on its function.

- To narrow the extensive X chromosomal modifier region with genetic mapping. Due to its size several modifier genes may potentially coexist in it. In case the region is significantly narrowed, the number of possible candidate genes also decreases. If this reaches a level when only a few genes are found on the specific chromosome section, studying both their sequence and expression may be targeted.

- If any sequential or expression difference can be identified during the work, then it is necessary to develop a special detection method to investigate it - one that can be used extensively and at population level.

2. MATERIALS AND METHODS

2.1. Bioinformatic studies

For the *in silico* (electronic) studies of my work I used genome databases (NCBI: http://www.ncbi.nlm.nih.gov/; Ensembl: http://www.ensembl.org/; MGI: http://www.informatics.jax.org/), primer designers (SeqVerter, GeneStudio Inc. 1999; Primer3, Rozen and Skaletsky 2000) and sequence-analyser programme packages (BioEdit, Hall 1997-2004; SeqVISTA, Hu et al. 2003; EMBOSS, Rice et al. 2000).

2.2. Experimental animals

2.2.1. Experimental animals used in the sequence and expression studies of the androgen receptor gene

For this research I have used the inbred strain developed from the Comp9 line (Figure 1A) and the CAST/Ei inbred strain (Figure 1 B). The Comp9 line was created by Dr Géza Müller (during sib-mating and selection for sixteen generations) from the original Compact strain that derives from Germany. This is a hypermuscled line homozygous for the *Mstn*$^{Cmpt\text{-}dl1Abc}$ deletion: swallow-bellied, large statured, but less massive built. The CAST/Ei strain was created during inbreeding from the mouse *Mus musculus castaneus* subspecies (www.informatics.jax.org). The single organisms are wild agouti coloured, remarkably small in size, normal muscled, are very lively and sensitive to environmental stress.

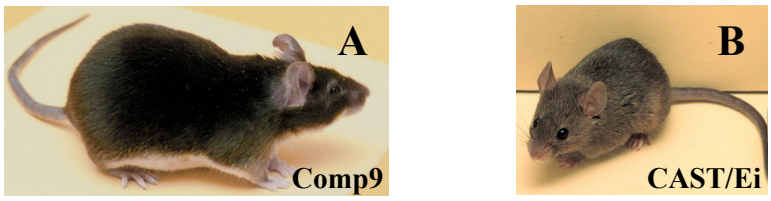

Figure 1 Mouse strains used for the sequence and expression studies of the androgen receptor gene; A: an individual of the Comp9 strain, B: An individual of the CAST/Ei strain (Varga et al. 2003b)

For the sequence and expression studies I used the samples of three hypermuscled (Comp9) and of three normal muscled control (CAST/Ei) male individuals (6-7 weeks old).

2.2.2. Experimental animals used for narrowing the modifier interval found on the X chromosome

During previous studies our research group created an intersubspecific cross called Cross4 from the Comp9 inbred line homozygous for the $Mstn^{Cmpt\text{-}dl1Abc}$ deletion and from the CAST/Ei (*Mus musculus castaneus*, wild type subspecies) inbred strain. The reason to include CAST/Ei in the formation of crossed populations and to employ it for genetic mapping is that as a subspecies genetically it is located farther as compared to other laboratory mouse strains, but it is fit for breeding during crossing. This provides a greater possibility for genetic marker polymorphism, which makes mapping more efficient. With the Cross4 (Comp9 male x CAST/Ei female) F2 population the significant myostatin modifier effects could be roughly positioned and linked to chromosomes (on chromosome 1, 3, 5, 7, 11, 16 and X) by our research group. Afterwards our group created a special mapping population that enables us to narrow the intervals which contain the modifier(s) on the highlighted chromosomes. This special mapping population created with complex crossing scheme is Advanced Intercross Lines (Darvasi and Soller, 1995), i.e. AIL, which we modified to Compact-AIL in our case. Compact-AIL has its origins in the Cross4 F2 population that was created for the mapping of the modifier genes. During the process the aim is to create successive breeding generations whilst maximally avoiding inbreeding. To achieve a higher mapping power we selected the Compact-AIL breeding generation in a way to have an F11 generation (mapping population) that is entirely homozygous for the $Mstn^{Cmpt\text{-}dl1Abc}$ mutation. As a result, a greater portion of the population (32%) will be suitable for mapping. Since the AIL breeding method increases the probability of recombination events, as a result the genome of the basic lines is significantly fragmented, which allows fine mapping (Pinke et al. 2008).
For the genetic analysis of the X chromosome we studied normal and hypermuscled (6-7 weeks old) animals of the Compact-AIL F11 generation. Due to the inactivation phenomena of the X chromosome we only use the phenotype-wise extreme groups of male animals: altogether 155 M1K and 248 M5K individuals (M = male, 1 = individual with normal muscles, 5 = hypermuscled individual, K = homozygous $Mstn^{Cmpt\text{-}dl1Abc}$ mutant). We created the technical groups summarized in Table 1 during genotyping necessary for mapping the X chromosome from extreme male F11 offsprings.

	M1K	M5K	Total per group:
G1 group	33	77	110
G2 group	61	59	120
G3 group	24	96	120
G4 group	37	16	53
Total:	155	248	403

Table 1 Compact-AIL F11 experimental groups used for genetic mapping (G: group). M1K: Normal muscled, M5K: hypermuscled individuals

The 403 animals in the study were selected from the Compact AIL-F11 generation consisting of 3100 individuals (Pinke et al. 2008) after visual review (Varga et al., 2002).

2.3. Sample preparation

We prepared the genomic DNA samples used during the investigation (Comp9, CAST/Ei and Compact-AIL F11) with saline precipitation method.
For my gene expression experiments I have prepared mixed skeletal muscle from the posterior limb of animals belonging to the Comp9 and to the CAST/Ei mouse strains (3-3 animals, 6-7 weeks old male individuals) without muscle preference. I have prepared the RNA samples from the tissue samples by using TRIZOL (GIBCO) reagent. From the RNA samples I have prepared cDNA-s with reverse transcription using the ProSTAR Ultra HF RT-PCR System kit of the Stratagene company, and I used these cDNA-s both during sequencing and during gene expression studies. I used the DNA samples for sequencing and population studies.

2.4. Sequence determination

I performed Comp9 and CAST/Ei Ar sequencing in two parts by using cDNA and genomic DNA templates. In order to determine the sequence I cloned the cDNA and DNA templates (pGEM®-T Easy), then I used the Applied Biosystems ABI PRISM BigDye® Terminator v3.1 Ready Reaction Cycle Sequencing kit according to the manufacturer's recommendation. To determine the Ar sequence of the 3-3 individuals for each strain (Comp9 and CAST/Ei) I used the ABI PRISM 310 Genetic Analyzer device.

2.5. Gene expression studies

During real time quantitative PCR experiments we studied the relative gene expression (androgen receptor gene; GAPDH and β-actin, as household genes) of 3-3 male individuals of mouse strains with different phenotypes (CAST/Ei and Comp9), in a three fold dilution (1-fold, 4-fold and 16-fold) and in three repetitions per each dilution. For the gene expression study I used the SYBR Green (Molecular Probes) method, the reactions ran on the ABI 7000 machine. I performed mathematical processing of the real time PCR results according to the Pfafll method, with relative quantification modified with efficiency correction and endogen control gene normalization (Pfaffl, 2001, Pfaffl et al 2002). I used the REST (relative expression software-tool) program for data analysis.

2.6. Genetic mapping

2.6.1. Genetic markers and marker development

We included the framework markers (FWM) seen on Figure 2 in the studies with the specific physical map positions (physical position in Mbp based on Ensembl Built34 and marker name; bold = the specific marker was a framework marker also in the Cross4 F2 mapping).

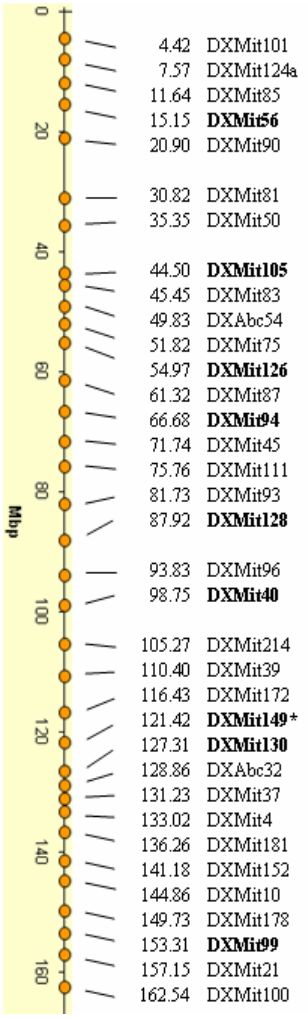

Figure 2 X chromosome microsatellite markers used during genetic mapping (framework markers: FWM). The common 9 markers of Cross4 F2 and Compact-AIL F11 are bold. * The DXMit149 marker was included instead of the DXMit116 marker. Next to the markers their physical position is indicated in Mbp (Ensembl Built34)

For the development of markers indicated with Abc (DXAbc54 and DXAbc32) we used the program package mentioned already in the Bioinformatic studies section,

and we used the mouse genome sequences requested from the www.ensembl.org online genome database.

2.6.2. The microsatellite detection method used during the study

Microsatellite marker detection was performed with polyacrylamide gel electrophoresis and silver staining method (Budowle et al. 1991, Varga et al. 1997).

2.6.3. Data management during the study

During the Compact-AIL F11 genetic mapping we expected the genetic effect that increases hypermuscularity from the elements of the Compact genome, and we expected the formation of normal muscles from the elements of the CAST/Ei genome according to the phenotype. Thus in the M5K (hypermuscled) animals we considered favourable the Comp9 allele acting towards hypermuscularity, while in the M1K animals the CAST/Ei allele was considered favourable that allows for the development of normal muscularity. The M5K-CAST/Ei and M1K-Comp9 phenotype-genotype combinations are not favourable according to our theory. In case of markers coupled with effective mutations the distribution of phenotype-genotype combinations changes significantly, i.e. in such cases the frequency of the Comp9 allele is significantly higher than that of the CAST/Ei allele in individuals with extreme muscularity (M5K) and vica versa. The highest modifier effect was therefore expected for those markers where the M5K group carried the Comp9 allele and the M1K group carried the CAST/Ei allele with a significantly higher frequency. Accordingly, the allele frequency (genotype frequency) values of the M1K and M5K individuals included in the study were merged:

- Favourable phenotype-genotype combinations
  FAV= M5K-Comp9 + M1K-CAST/Ei

- Unfavourable phenotype-genotype combinations
  UNFAV= M5K-CAST/Ei + M1K-Comp9

The Chi^2 test was performed in a way that we compared the FAV and UNFAV values obtained for a specific marker with the expected equal allele frequency values. As a result of this we obtained the Chi^2 curve demonstrating the modifier effect. This curve presents the Chi^2 values of the markers on a logarithmic scale along the X chromosome.

3. RESULTS

3.1. Studying the androgen receptor gene of the Compact mouse

3.1.1. Studying the androgen receptor gene sequence in Compact mice and in normal muscled mice

Due to the significant genomic size of the Ar (167.4 kb) as a first step I determined the cDNA sequence that contains the coding regions of the gene. However, I could not translate the entire cDNA from the mRNA independently of gender and strain. Thus, sequence determination was a 2 step process. First I determined the sequence of the cDNA region that contains exons 2 to 8 of the Ar gene, then I analyzed exon1 amplified from the genomic DNA. This is how finally the sequence of the entire coding part of the Ar gene could be studied. After sequencing the three-three independent samples per strain, I found four SNP-s (single nucleotide polymorphism) in the CAST/Ei Ar sequence. These mutations can be found in the www.jax.org online SNP database under the following rs (reference SNP) identifiers: rs31851337 (at codon 324), rs29087626 (at codon 346), rs31851336 (at codon 388) and rs29085429 (at codon 715l). From among the SNP-s the rs31851337 and the rs29087626 causes amino acid exchange. Thus at codon 324 there is an asparagin instead of serine, and at codon 346 there is isoleucine instead of leucine in the CAST/Ei androgen receptor protein. These amino acid exchanges are found in exon 1 that encodes the transcription regulating amino-terminal domain of the protein. The Comp9 coding Ar sequence was entirely the same as the reference Ensembl C57Bl/6 sequence.

3.1.2. Gene expression study of the androgen receptor gene in Compact mice and in normal muscled mice

I investigated the expression of the androgen receptor gene with real time quantitative PCR reaction. In my studies I used the 64 base-pair sequence overlapping exon 1 and exon 2. I expressed the extent of the Comp9 and CAST/Ei Ar gene expression relative to each other. During the studies I found no deviations indicating multiple gene expression between the unit CAST/Ei expression and the Comp9 Ar expression compared to it.

3.2. Narrowing the chromosome section that influences hypermuscularity and that is located on the X chromosome of the Compact mouse

3.2.1. Genetic mapping on the F11 generation

For mapping the gene or genes influencing hypermuscularity and located on chromosome X, I studied those homozygous mutant ($Mstn^{Cmpt\text{-}dl1Abc}/Mstn^{Cmpt\text{-}dl1Abc}$)

animals of the Compact-AIL experimental population F11 generation that represented extremes with regards to hypermuscularity, i.e. normal (M1K) and hypermuscled (M5K) phenotype individuals. In order to evenly cover the X chromosome on an average distance of 4,94 Mb (highest and smallest distance: 6,52 Mbp and 3,15 Mbp) per marker I used the microsatellite markers in the Ensembl (www.ensembl.org) online database (DXMit) and microsatellite markers designed by us based on the database sequence (DXAbc). The marker set included the previous 9 microsatellite markers that we studied in F2. Hereinafter I mention them under the common name of framework markers. I started to genotype the framework markers from the DXMit 128 marker that gave the highest LOD value based on the earlier F2 results, and I proceeded from here both distally and proximally. FWM-s were first genotyped only to the G1 and G2 groups. Altogether this meant 94 normal muscled and 136 hypermuscled, homozygous mutant males. As a first step we examined these 230 individuals for 31 FWM markers. As a result of more than 7000 genotypings we detected a strong effect influencing hypermuscularity on the X chromosome at 49,83 Mbp for the DXAbc54 microsatellite and at 131,23 Mbp for the DXMit37 microsatellite (Figure 3; indicated with a yellow circle).

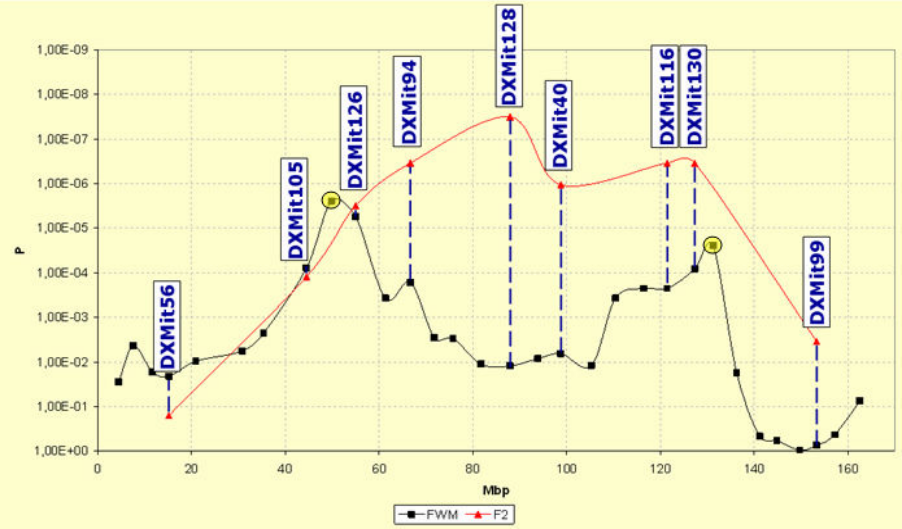

Figure 3 X chromosome F11 (n=230) mapping as compared to Cross4 F2 (n=85) results. F2 marker number: 9 (red triangle), F11 marker number: 31 (black square). Common markers are connected with blue dotted line. The two markers indicated with yellow circle showed significant modifier effect during the F11 mapping

The FAV-UNFAV (see the section called Data management during the study) joint action curve obtained with Chi^2 method has shown strong, significant 2,45E-06 and 2,44E-05 values for these points.

3.2.2. Further narrowing of modifier regions found in the F11 generation

In the case of both peaks (DXAbc54; at 49,83 Mbp and DXMit37; at 131,23 Mbp) we included 1-1 further marker both proximally and distally (Figure 4, indicated in green) to narrow the strongest modifier effect. These markers around the DXMit37 are: the DXMit4 (distal) and the DXAbc32 (proximal); while around the DXAbc54: the DXMit75 (distal) and the DXMit83 (proximal). At this stage we increased the number of animals to be studied in order to have even more precise data, i.e. besides the G1-G2 offspring groups (a total n=230) we also included G3-G4 groups (total n=173) in the genotyping. Thus this study already included altogether 403 Compact-AIL F11 individuals (155 M1K and 248 M5K). As a result of a further almost 2000 genotypings the Chi^2 value decreased at the DXMit4 marker that was included on the distal side of the DXMit37 marker, while we obtained a value of 1,41E-06 for the proximally positioned DXAbc32 marker. This point (DXAbc32) is the highest peak of the so-called XA region bordered by the DXMit130-DXMit37 markers. In case of the DXAbc54 marker the Chi^2 value also decreased on the distal side for the DXMit75 marker, while it increased to a value of 8,55E-09 for the proximally positioned DXMit83 marker. This is currently the highest point (DXMit83) of the XB region bordered by the DXMit105 and DXAbc54 markers.

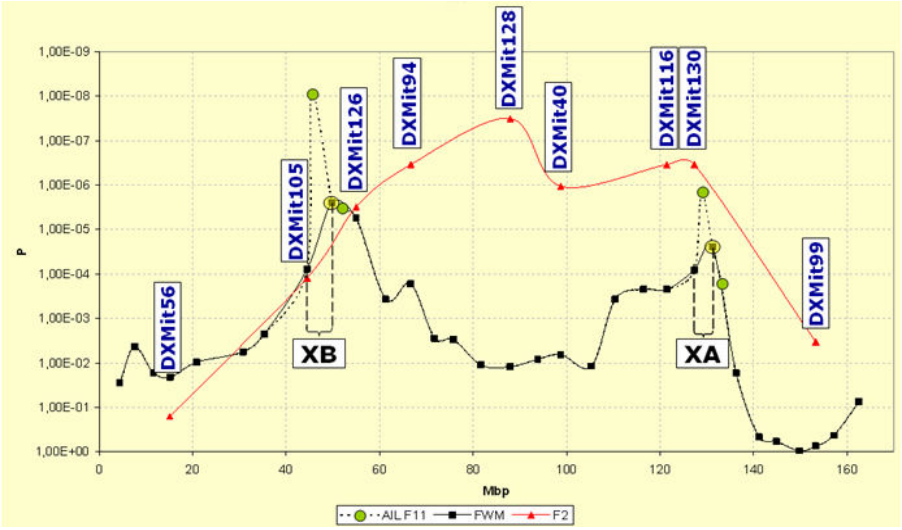

Figure 4 Narrowing the modifier intervals of the X chromosome. During F11 mapping besides the markers showing significant modifier effect (yellow circle) we included additional markers (green circle), which were examined with a higher number of animals (n=403) and we determined two candidate intervals (XA and XB)

3.2.3. Determining two highly significant modifier regions

In order to narrow the two adjacent modifier regions we included further individuals of the mapping population and examined newer markers. Thus we

defined two strong acting and well definable intervals, which we named XA and XB. The length of XA is 3,92 Mbp and includes approximately 60 genes (or suspected genes) according to Ensembl Built34 data. The length of the XB region is 5.33 Mbp and the location of approximately 30 genes (or suspected genes) is known.

3.3. New scientific results

During my work the following new scientific results were revealed:

1. During my sequencing investigations in the Comp9 myostatin mutant mouse strain I determined the cDNA sequence of the androgen receptor gene (Ma et al. 2001, Varga et al 2003a) considered to be a candidate myostatin modifier. The Ar gene showed no deviation relative to the wild mouse strain.

2. During the investigation of the population that was created with a special crossing procedure (Compact-AIL) I divided the myostatin modifier region located on the X chromosome - that was identified during a previous F2 analysis - into two modifier regions, thus verifying the special resolution power of the AIL population. I proved that the research in F2 revealed a so-called ghost-QTL (see the Conclusions and recommendations section) as a point indicating the strongest linkage, which presented as a joint dummy effect of the two adjacent real QTLs. Through inclusion of further microsatellite markers I managed to determine two narrow, well definable modifier regions on the X chromosome that I named XA and XB.

3. I established that the androgen receptor gene is not likely to be involved in the development of the hypermuscled phenotype of the Comp9 mouse strain, since it is not located in the narrowed XA and XB regions, and neither its sequence, nor its expression differs from that of the wild type.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. The androgen receptor gene as candidate gene

During my scientific investigation I studied the androgen receptor gene due to its position (based on an F2 mapping population analysis, its genomic position is in the vicinity of the DXMit128 marker which indicates the peak of the X chromosomal modifier effect; Varga et al. 2003a) and role in muscle development (Ma et al. 2001, Chipuk et al. 2002) as a candidate myostatin modifier.

4.1.1. Sequence study of the androgen receptor gene

Due to the extensive genomic size of the gene I first determined its coding sequence in three Comp9 (hypermuscled) and in three control CAST/Ei (normal muscled) mice. Based on the results I compared the Comp9 Ar coding sequence with the consensus sequence (Ensembl C57Bl/6) and with the Cast/Ei sequence. We found no Comp9 specific mutation in the Ar gene. We could only detect the already known four CAST/Ei SNP-s in the inbred strain created from the *Mus musculus castaneus* subspecies. Only two of the four mutations cause amino acid exchange, the rs31851337 at codon 324 and the rs29087626 at codon 346. These changes that cause amino acid exchange are found in the transcription regulatory part of the CAST/Ei protein. Thus if these mutations affect the phenotype then in case of the CAST/Ei strain it may be revealed in the regulatory function of the Ar protein exerted on other genes' transcription. Since the CAST/Ei strain shows no extreme muscled characteristics, it is likely that CAST/Ei Ar SNP-s do not play a role in the development of the hypermuscled phenotype. We can also establish that presumably the detected, amino acid exchanging SNP-s are subspecies-type characteristics, since these mutations cannot be found in any of several inbred mouse genomes, nor in the consensus C57B1/6 genomic sequence.

4.1.2. Gene expression study of the androgen receptor gene

During this research we determined the relative amount of Ar RNA in case of 6-7 weeks old male Comp9 (hypermuscled) and CAST/Ei (normal muscled) mice. The Comp9 Ar relative expression (1,16) proved approximately identical with the CAST/Ei Ar expression regarded as a single unit. Taking the P-value of 0.001 into account, this minor difference can be considered significant. However, in total the quantitative analysis of gene expression did not show significant differences between the skeletal muscle Ar mRNA levels of Comp9 (hypermuscled) and CAST/Ei (normal muscled) male mice.

4.1.3. Reevaluation of the androgen receptor gene role as a candidate gene

Ar proved to be suitable for the positionally candidate modifier gene role regarding its nearby position to the point showing the strongest effect based on the modifier region mapping performed on the F2 generation resulting from the Cross4 crossing (Comp9 x CAST/Ei), as well as regarding its function. However our previous results show that neither the coding sequence nor the expression level of the mutant Ar underlies its myostatin (*Mstn*$^{Cmpt\text{-}dl1Abc}$) modificator role (Veress et al. 2009). Possible micro RNA regulation of the Compact Ar could have been studied at the protein level by further methods, since some miRNAs may affect the Ar signal transduction pathway (Epis et al. 2009). As the high-resolution mapping population was available shortly after our preliminary examinations, our research group decided to carry out these time consuming, costly examinations only if the mapping results of the special population confirmed the Ar's candidate role.
Thus our main target was switched to narrow the X chromosomal modifier region instead of searching for and investigating further candidate genes.

4.2. Genetic mapping in the Compact-AIL F11 generation

4.2.1. Comparing the Compact-AIL F11 and the Cross4 F2 results

After more than 7000 genotypings we had the opportunity to compare the Compact-AIL F11 results with those obtained in Cross4 F2 Results have shown that while there the highest values were obtained around 90 Mbp, here in the same position the value was below the significance level. In addition, this result verified our previous assumption that instead of one, there are two adjacent modifier regions of strong effect located on the X chromosome (Figure 5). The Compact-AIL investigation was therefore suitable for me to dissociate this dual effect, proving that in fact there is no significant effect at the location specified by the F2 analysis, and this is a so-called ghost-QTL (Haley and Knott 1992, Martinez and Curnow 1992).

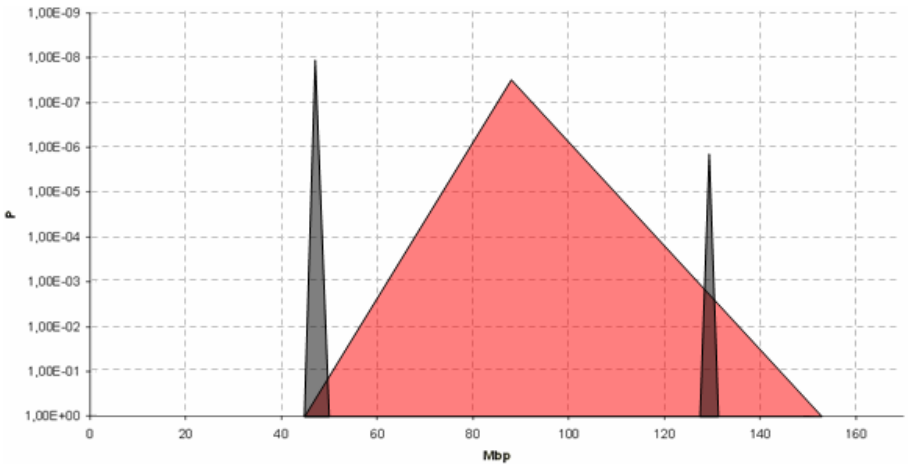

Figure 5 Comparison of the X chromosome F11 (n=230) mapping results with the F2 (n=85) results. The red triangle shows the F2 mapping result, while the two black triangles show the F11 mapping result. On the left side of the figure the level of significance is seen as expressed in P-value, while the scale below the figure shows the physical distance of chromosomal points in megabases from the proximal end towards the distal end).

Ghost-QTL indicates an unreal quantitative trait locus (QTL), which can be detected, if there is/was not enough recombination between two or more mutations having an effect on the given phenotype, and thus the two effects are jointly detected on a more extended section. Since only a few recombinant events happen until the F2 generation (mainly regarding the X chromosome), therefore this is a reasonable explanation of the results experienced in Cross4.

4.3. A step forward in the mapping of X chromosomal modifier genes

During our work we used a special mapping population derived via a complex crossing scheme (Pinke et al. 2008) to narrow an approximately 100 Mbp modifier region. Due to the accumulation of recombination events a population like this is the most feasible tool for fine mapping. For its high resolution power the efficiency can be multiple to that of the F2.
In the course of genetic mapping on the Compact-AIL F11 population, modifier effect was not detected neither at the DXMit 128 microsatellite marker at the previous F2 peak nor at the additional new markers – due to recombination.
Proceeding with genetic mapping two adjacent modifier sections were detected and further narrowed by involving additional markers. These regions were denoted with XA and XB. Within, further recombination might be detected allowing for further narrowing that in case of appropriate recombination frequency can lead to the identification of the effective mutation.
Accordingly designating and testing of other genes with different methods following costly, time consuming optimization of experimental set-ups was not an aim.

Thus any potential further X chromosomal modifier studies should focus only on these 3.92 Mbp and 5.33 Mbp sections instead of the extended region (approx. 100 Mbp) already known from the F2, which is a great step forward in every respect.

4.4. Further potentials of fine mapping

Due to the crossing started with CAST/Ei several further informative markers can be found both in the XA and in the XB interval in accordance with the prior polymorphism studies. As a consequence of the recombinant events accumulated in the Compact-AIL crossing there is good chance to narrow these regions further, which greatly supports mapping of hypothetic modifiers. In case this process reaches a few-gene level, it is reasonable to study these genes both at the level of sequence and expression. If during the work any sequential or expression difference can be detected, then is must be verified on population level as well. A special detection method that can be used extensively will need to be developed in order to accomplish this. However, it is not evident at all that the mutation leading to the modifier effect is located in a gene.

PUBLICATIONS RELATED TO THE DISSERTATION

Scientific papers:

Veress Gy., Bakos K., Korom E., Pinke O., Kovács B., Varga L. (2009): Sequence and expression analysis of the androgen receptor gene from Compact mouse. *Archiv für Tierzucht*, 2009/52, Issue 2, 212-214. IF: 0,679 (2008)

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