# *MYOSTATIN* GENE POLYMORPHISM AND DOUBLE MUSCLING EXPRESSION IN CATTLE BREEDS : PRELIMINARY RESULTS

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## INTRODUCTION

The bovine muscular hypertrophy (***mh***) syndrome occurs at different frequencies in many European cattle breeds. The phenotype mainly implies an increased muscle development, responsible of the name *double muscling*, increased growth rate and carcass value (Ménissier 1982; Arthur 1995). Several mutations in the *myostatin* gene were described as the genetic cause (Grobet *et al*., 1998) and later, *myostatin* haplotypes were described (Miranda *et al*., 2000; Dunner *et al*., submitted). Using an OLA assay (Grossman et al., 1998) animals belonging to different beef populations have been genotyped for *myostatin* gene mutations and also externally evaluated (Neuvy and Vissac, 1962) in order to be able to determine associations between genotypes and particular characters involved in the double-muscling trait.

## MATERIAL AND METHODS

**Biological material.** 2042 calves were randomly sampled among eight European beef cattle breeds: three Spanish: *Asturiana de los Valles* (AV), *Pirenaica* (PI) and *Rubia Gallega* (RG), and five French breeds : *Bazadaise* (BZ), *Charolaise* (CH), *Gasconne* (GA), *Maine-Anjou* (MA) and *Parthenaise* (PA). Individuals were scored for three phenotypic groups of traits attending double muscling character: 1) **General Body Conformation** (*animal general classification, muscling depth, back width*), 2) **Muscle Expression** (*shoulder muscle depth, longissimus dorsi and great dorsal hypertrophy, thigh circumference, croup slope, inter-muscular definition*) and 3) **Hypertrophy Associated Traits** (*bone size, tail position, posture and gait, abdomen shape, skin type*). Each of them was obtained from 0 (no hypertrophic signs) to 2 (high double muscling) scoring. The three notes were added to obtain a Total Phenotypic Scoring (TPS) ranging from 0 to 28.

**Genotyping myostatin mutations.** An OLA assay (Miranda *et al*., submitted) was here applied, detecting the nine mutations affecting the coding sequence of the *myostatin* gene ; five disrupting the protein (nt821, Q204X, E226X, C313Y, nt419), one conservative mutation (F94L) and two missense (S105C and D182N; results not shown). Genotypes are scored as haplotypes, each corresponding to the combination of none, one or two mutations.

**Statistical Analysis.** The frequencies of the found genotypes were calculated within breeds. An Analysis of Variance was conducted using the GLM procedure of SAS (SAS, Inst. Inc.

Cary, NC) for the main effect of genotype within breeds. The scores of all phenotypic traits were used as variables to cluster the genotypes using Correspondence Analysis.

## RESULTS AND DISCUSSION

**Myostatin gene genotypes.** Table 1 shows the genotypes frequencies. All observed genotypes are in agreement with the *myostatin* haplotypes previously identified (Miranda *et al*., 2000 ; Dunner *et al*., submitted) and also confirm that some mutations (disruptive nt821 and conservative F94L) are wide-spread in bovine populations. However, other mutations supposed exclusive of a breed by previous studies (E226X, nt419 and Q204X ; Grobet *et al*., 1998), are detected in other breeds.

**Table 1. Frequencies (in %) of genotypes within breeds.** Genotypes are different coloured attending to the number of haplotypes showing disruptive mutations (dark grey : two ; medium grey : one ; and light grey : none).

| Genotypes | AV | BZ | CH | GA | MA | PA | PI | RG |
|---|---|---|---|---|---|---|---|---|
| nt821-nt821 | 74.0 | | | | | 77.1 | 1.4 | 28.8 |
| Q204X-Q204X | | | 33.3 | | | | | |
| E226X-E226X | | | | | 17.4 | 0.4 | | |
| C313Y-C313Y | | | | 10.3 | | | | |
| nt821-E226X | | | | | | 10.1 | | |
| Q204X-nt821 | | | 0.5 | | | 1.9 | | |
| nt419-E226X | | | | | 9.8 | | | |
| nt821-nt419 | | | | | | 4.4 | 0.2 | |
| nt419-F94L | | | | | | | 1.9 | |
| nt821-F94L | 0.4 | | | | | 1.5 | 22.1 | 0.9 |
| Q204X-F94L | | | 3.3 | | | | | |
| C313Y-F94L | | | | 0.3 | | | | |
| nt419-None | | | | | 8.6 | | 0.7 | |
| nt821-None | 23.5 | | | | | 3.0 | 5.5 | 61.4 |
| Q204X-None | | | 14.3 | | | | | |
| E226X-None | | | | | 37.3 | 0.4 | | |
| C313Y-None | | | | 59.0 | | | | |
| F94L-F94L | | | | | | 0.7 | 21.4 | |
| F94L-None | | | 5.7 | | | | 33.2 | 0.9 |
| None-None | 2.1 | 100 | 42.9 | 30.3 | 26.9 | | 13.5 | 8.2 |
| Total Number of individuals | 238 | 47 | 210 | 300 | 327 | 271 | 416 | 233 |

**Analysis of Variance.** The results corresponding to TPS means for all genotypes are shown in Table 2. In most breeds TPS mean is significantly different when two disruptive mutations are present. This is not true for PA, PI and RG breeds, probably caused by a deficient phenotypic scoring in these breeds.

**Table 2. Effect of Genotype on Total Phenotypic Scoring.** Genotypes are different coloured attending to the number of haplotypes showing disruptive mutations (dark grey : two ; medium grey : one ; light grey : none). Only results for TPS means are shown. Means within breeds with a different superscript differ (P<0.0001).

| Genotypes | AV | BZ | CH | GA | MA | PA | PI | RG |
|---|---|---|---|---|---|---|---|---|
| nt821-nt821 | 19.1$^{a}$ | | | | | 8.6$^{a}$ | 4.6$^{bc}$ | 14.8$^{a}$ |
| Q204X-Q204X | | | 23.0$^{a}$ | | | | | |
| E226X-E226X | | | | | 18.6$^{a}$ | 8.5$^{a}$ | | |
| C313Y-C313Y | | | | 23.0$^{a}$ | | | | |
| nt821-E226X | | | | | | 8.5$^{a}$ | | |
| Q204X-nt821 | | | 22.0$^{a}$ | | | 10.9$^{a}$ | | |
| nt419-E226X | | | | | 18.8$^{a}$ | | | |
| nt821-nt419 | | | | | | 6.5$^{a}$ | 0.5$^{c}$ | |
| nt419-F94L | | | | | | | 7.7$^{ba}$ | |
| nt821-F94L | 16.0$^{ab}$ | | | | | 0.9$^{a}$ | 6.7$^{bac}$ | 14.0$^{a}$ |
| Q204X-F94L | | | 18.4$^{a}$ | | | | | |
| C313Y-F94L | | | | 3.0$^{b}$ | | | | |
| nt419-None | | | | | 4.5$^{b}$ | | 12.2$^{a}$ | |
| nt821-None | 5.0$^{c}$ | | | | | 4.4$^{a}$ | 6.8$^{bac}$ | 13.9$^{a}$ |
| Q204X-None | | | 6.5$^{b}$ | | | | | |
| E226X-None | | | | | 4.2$^{b}$ | 9.0$^{a}$ | | |
| C313Y-None | | | | 2.0$^{b}$ | | | | |
| F94L-F94L | | | | | | 3.5$^{a}$ | 4.9$^{bc}$ | |
| F94L-None | | | 2.5$^{b}$ | | | | 4.4$^{bc}$ | 12.5$^{a}$ |
| None-None | 10.2$^{bc}$ | 13.0 | 1.7$^{b}$ | 0.6$^{b}$ | 2.7$^{b}$ | | 6.0$^{bac}$ | 14.1$^{a}$ |
| TPS Mean | 16.18 | 13.03 | 10.27 | 3.76 | 7.74 | 8.25 | 5.48 | 14.13 |

**Correspondence Analysis.** Axis 1 contributes 70% of the total inertia and separates individuals carrying haplotypes containing two disruptive mutations from those which do not (Figure 1a). The contribution of the most important phenotypic traits are also represented in Figure b .

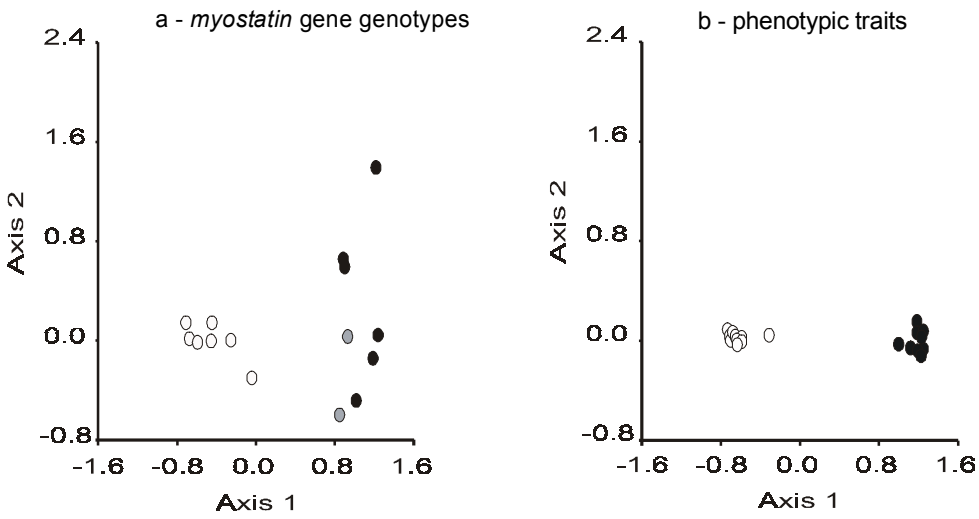


**Figure 1. Correspondence Analysis. a.** Genotypes are represented as spots coloured attending to the mutations present (black: two disruptive mutations; grey: one disruptive mutation in combination with F94L mutation; white: no disruptive mutation). **b.** Most influential phenotypic variables contributing to Axis 1 and 2 are represented as different coloured spots (white : 0 scored categories . black : 2 scored categories). (Data from PA, PI and RG breeds are not included).

## CONCLUSION

In most breeds homozygotes and double heterozygotes for disruptive mutations have significantly higher phenotypic scorings. Although higher, heterozygotes for one disruptive mutation do not show significantly different scoring from those lacking any disruptive mutation. Heterozygotes for either disruptive mutation (nt821, Q204X) in combination with F94L mutation show higher significant phenotypic scores than wild-type individuals.

## REFERENCES

Arthur, P.F. (1995) *Aust. J. Agric. Res.* **46** : 1493-515.

Grobet, L., Poncelet, D., Royo, L.J., Brouwers, B., Pirottin, D, Michaux, C., Ménissier, F., Zanotti, M., Dunner, S. and Georges, M. (1998) *Mammalian Genome* **9** : 210-213.

Grossman, P.D., Bloch, W., Brinson, E., Chang, C.C., Eggerding, F.A., Fung, S., Lovannisci, D.A., Woo S. and Winn-Deen E.S. (1994) *Nucleic Acids Research* **22** : 4527-34.

Ménissier, F. (1982) *Curr. Top. Vet. Anim. Sci.* **16** : 23-53.

Miranda, M.E., S. Dunner, Y. Amigues, M.-Y. Boscher, F. Bourgeois-Bossaert, J. Cañón, O. Cortés, M. Georges, L. Grobet, R. Hanset, P. Maugrion, and F. Ménissier. (2000). Proc 27th ISAG 2000 : 93.

Neuvy A. and Vissac B. (1962) «Union National des Livres Généalogiques», Paris : 52.