Genetic screening of the inherited Ichtyosis causative mutation in Chianina cattle

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ABSTRACT - Inherited Ichthyosis is a genetic disorder reported in both humans and animals, including bovines. Two inherited forms were reported in cattle and both are transmitted in an autosomal recessive manner: Ichthyosis Fetalis (IF) and Ichthyosis Congenita (IC). A causative mutation of IF in Chianina cattle was recently indentified in the ABC12 gene. This work reports the first genetic screening using this recently available genetic test on Chianina cattle. Tests were performed on both the population of farm breeding selected young bulls (131 samples randomly chosen) and high breeding value sires (16 samples). Results confirm a low total prevalence of carriers in the selected sire population (2/131; 1.5%) and the presence of the disease allele among the high value selected sires (1/16; 6.3%). This result strengthens the importance to continue the genetic screening program, particularly in performance tested bulls approved for use in AI or natural service.

Key words: Inherited Ichthyosis, Chianina, Causative mutation, Genetic screening.

Introduction - Inherited Ichthyosis is a rare keratinisation disorder characterized by an excessive amount of superficial scale on the skin. In cattle, it is always present at birth and two main clinical forms 1) Ichthyosis Congenita (IC) and 2) Ichthyosis Fetalis (IF) were reported. IC is the less severe and localized form and it is often compatible with life (Baker and Ward, 1985; Yager and Scott, 1993; Raoofi *et al.*, 2001; Testoni *et al.*, 2006). IF, reminiscent of Harlequin Ichthyosis in humans, is the extreme clinical form. Affected calves show cutaneous lesions, with deep fissures separating hyperkeratotic skin plaques and eversion of mucocutaneous junctions. It is always fatal and affected animals are aborted or they survive only a few days after birth (Molteni *et al.*, 2006; Cho *et al.*, 2007).

IF severe form is inherited as simple autosomal recessive trait, and it was reported in Chianina cattle breed (Molteni *et al.*, 2006).

In a pioneering use of tens of thousands of SNP markers combined with the typical structure of a bovine pedigree, the causative mutation of IF in Chianina cattle was recently identified in the ABCA12 gene (Charlier *et al.,* 2008). This gene encodes the 12th member of the A subfamily of ATP-binding cassette transporters and the normal form of this peptide is conserved in all known vertebrate sequences. These findings have had an immediate application about the breeding practice.

Due to the high market value of Chianina calves and, therefore, the possible economic loss caused by Ichthyosis, this study has aimed to identify IF mutation carriers in Chianina breed. Based on controls on performance tested and breeding population stud bulls, this first report provides indication on prevalence of IF in the breed. Breeding strategies to eradicate the defect are also considered.

Material and methods – During 2008, a total number of 147 healthy purebred Chianina bulls were genotyped for the IF disease mutation. Blood samples were obtained from 16 performance tested young bulls at the Genetic Center of the Italian Beef Cattle Breeders' Association (ANABIC) and from 131 young bulls randomly chosen within the selected for breeding in more than 60 different farms under the supervision of ANABIC (Table 1).

DNA was extracted from whole peripheral blood in EDTA through a standard extraction procedure. Gene genotype was detected by amplification, purification and sequencing (Longeri *et al.*, 2007). Electrophoresis of purified sequencing reactions was performed on an ABI PRISM 310 DNA analyzer (PE Applied Biosystems). Positive animals were re-analysed to confirm the presence of the disease mutation.

Results and conclusions – Advanced technologies will increase the diagnostic power in the next years and they will have important practical consequences, as they will allow the rapid control of emerging recessive defects with otherwise major economic and animal welfare implications. The availability of a genetic test is essential to eliminate a defect from a breed within a matter of months after the discovery of the causal mutation (Charlier *et al.*, 2008). In 2008, Chianina breeding selected young bulls numbered 511 (personal communication). The Genetic Screening on 147 Chianina males (about 28% of the total population of 511 future breeding young bulls) identified a total of 2.04% heterozygous carriers for the causative mutation. Considering the provenience of the samples, the prevalence increases from 1.5%, in the farm bulls, to 6.3% in bulls collected from the Genetic Center, indicating a higher presence of the disease allele in the high breeding value population (Table 1). As a matter of fact the history of this disease in the Chianina breed reveals that all clinical cases identified are related to a single common ancestor, a sire widely used in Artificial Insemination (Molteni *et al.*, 2006). An as low percentage as 6% of carriers in future high breeding value sires represents a danger for semen quality and semen exportation if not managed.

Table 1. Genetic screening of ABCA12 culprit exon in Italian Chianina young breeding bulls during 2008.

Origin	N. of genotyped bulls	N. of mutation- carrier bulls	% carriers
Farms	131	2	1.5%
ANABIC Genetic center	16	1	6.3%
Total	147	3	2.04%

The importance of avoiding IF carriers in future breeding needs not to be further discussed. Our analysis did not consider females, underestimating the presence of the disease allele in the population. It would be of importance to include in the genetic screening also high breeding females.

The prevalence in the total population analysed is under 3%, pointing out a rapid and successful eradication by excluding from mating the carriers without threatening breed genetic variability.

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