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# Short communication: Prevalence of deleterious variants causing recessive disorders in Italian Chianina, Marchigiana and Romagnola cattle

J.G.P. Jacinto [a,b], F. Sbarra [c], A. Quaglia [c], A. Gentile [a], C. Drögemüller [b,*]

[a] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, BO, Italy
[b] Institute of Genetics, Vetsuisse Faculty, University of Bern, Bremgartenstrasse109a, 3012 Bern, Switzerland
[c] National Association of Italian Beef-Cattle Breeders, Strada del Vio Viscioloso, 21, 06132 Perugia, Italy

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ABSTRACT

In the last two decades, the molecular cause of six monogenic autosomal recessive disorders has been identified in native Italian beef cattle: two different *ATP2A1* variants for the pseudomyotonia congenita, the first in Chianina and Romagnola (**PMT1**) and the second in Romagnola (**PMT2**); a *KDM2B* variant for the paunch calf syndrome (**PCS**) in Marchigiana and Romagnola; a *NID1* variant for the congenital cataract (**CC**) in Romagnola; a *LAMB1* variant for the hemifacial microsomia (**HFM**) in Romagnola; an *ABCA12* variant for the ichthyosis fetalis (**IF**) in Chianina and a *FA2H* variant for the ichthyosis congenita (**IC**) in Chianina. The aim of this study was to evaluate the potential impact of these disorders in the affected Italian populations. For this purpose, 3331 Chianina, 2812 Marchigiana and 1680 Romagnola bulls born in the last 40 years were considered. The allelic frequency (**AF**) of the variant for PMT1 was 1.0% in Romagnola, 4.6% in Marchigiana and 5.9% in Chianina. The AF of the variant for PMT2 was 3.3% in Romagnola and 0% in the other two breeds. The AF of the variant for PCS was 11.7% in Romagnola, 2.0% in Marchigiana and 0% in Chianina. The AF of the variants for CC, HFM, IF and IC resulted below 3%, being the variants detected only in the breed populations in which they were previously reported. Considering a selected male population in the single breed, Chianina showed carrier prevalence of 11.9% for PMT1, 7.7% for IC and 6.4% for IF. Romagnola showed carrier prevalence of 23.4% for PCS, 6.7% for PMT2, 4.1% for HFM, 3.2% for CC and 2.0% for PMT1. Marchigiana showed carrier prevalence of 9.1% for PMT1 and 4.0% for PCS. With respect to the Romagnola cattle, the concerning presence of a total of five defect alleles in the population hampers a general approach based on the prevention of carriers from artificial insemination. However, identification of carriers may allow conscious mating to prevent the risk of homozygous descendants as well as the spread of heterozygous offspring. Therefore, systematic genotyping for all seven known harmful alleles is recommended to prevent risk mating between carriers, in particular to avoid the occurrence of affected offspring.



## Implications

Deleterious variants are the most important factors contributing to genetic load and inbreeding increases the likelihood of offspring receiving two copies of harmful recessive alleles. Knowledge of the frequency of carriers of inherited disorders in cattle populations is an essential prerequisite for breeders' associations to adopt preventive measures to reduce the risk of further spread of undesired alleles. Moderate to high frequency of deleterious alleles in Italian beef cattle populations creates awareness of genetic disease occurrence. Systematic genotyping of male animals used for reproduction and subsequently labelling them as carriers is an effective approach to prevent the spread of deleterious alleles in the populations.

## Introduction

An increasing number of diseases and traits in cattle have been characterised at the molecular level, with the identification of the causative gene variant at the DNA level. This occurred also within Chianina, Marchigiana and Romagnola, the three main native Italian beef cattle breeds located in the Apennine Mountains. Although in the past, they were mainly used as draught animals, in recent

* Corresponding author.
*E-mail address:* cord.droegemueller@vetsuisse.unibe.ch (C. Drögemüller).

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**Table 1**
Overview of tested harmful alleles and occurrence in the total number of genotyped male cattle per breed.

| Disorder (OMIA) | Gene | Variant | Breed | N_Gen | AF |
|---|---|---|---|---|---|
| PMT (OMIA 001464-9913) | *ATP2A1* | p.Arg164His (PMT1) | Chianina | 3 331 | 0.059 |
| | | | Marchigiana | 2 812 | 0.046 |
| | | | Romagnola | 1 680 | 0.010 |
| | *ATP2A1* | p.Gly211Val (PMT2) | Chianina | 96 | 0 |
| | | | Marchigiana | 284 | 0 |
| | | | Romagola | 375 | 0.033 |
| PCS (OMIA 001722-9913) | *KDM2B* | p.Asp835Asn | Chianina | 96 | 0 |
| | | | Marchigiana | 378 | 0.020 |
| | | | Romagnola | 1 546 | 0.117 |
| CC (OMIA 001936-9913) | *NID1* | 855 bp deletion | Chianina | 96 | 0 |
| | | | Marchigiana | 115 | 0 |
| | | | Romagnola | 691 | 0.016 |
| HFM (OMIA 002479-9913) | *LAMB1* | p.Arg668Cys | Chianina | 96 | 0 |
| | | | Marchigiana | 96 | 0 |
| | | | Romagnola | 314 | 0.021 |
| IF (OMIA 002238-9913) | *ABCA12* | p.His1935Arg | Chianina | 3 330 | 0.032 |
| | | | Marchigiana | 209 | 0 |
| | | | Romagnola | 162 | 0 |
| IC (OMIA 002450-9913) | *FA2H* | 1 bp insertion | Chianina | 366 | 0.038 |
| | | | Marchigiana | 95 | 0 |
| | | | Romagnola | 94 | 0 |

*Abbreviations:* N_Gen = total number of genotyped male animals; AF = allele frequency; OMIA = Online Mendelian Inheritance in Animals; PMT = pseudomyotonia congenita; PCS = paunch calf syndrome; CC = congenital bilateral cataract; HFM = hemifacial microsomia; IF = ichthyosis fetalis; IC = ichthyosis congenita.

decades, they have been included in intensive selective breeding programmes to improve their performance for beef production leading to increased rates of inbreeding (Mastrangelo et al., 2018). These have resulted in the emergence of several Mendelian disorders. So far, six recessively inherited disorders have been studied in detail. The list includes (1) pseudomyotonia congenita (**PMT**) (OMIA 001464-9913); (2) paunch calf syndrome (**PCS**) (OMIA 001722-9913); (3) congenital bilateral cataract (**CC**) (OMIA 001936-9913); (4) hemifacial microsomia (**HFM**) (OMIA 002479-9913); (5) ichthyosis fetalis (**IF**) (OMIA 002238-9913); (6) ichthyosis congenita (**IC**) (OMIA 002450-9913).

PMT is characterised by exercise-induced muscular stiffness which prevents animals from performing rapid movements. Two different causative missense variants in ATP2A1 have been found: the first in Chianina (**PMT1**:p.Arg164His) and Romagnola (Drögemüller et al., 2008), the second in Romagnola (**PMT2**:p.Gly211Val) (Murgiano et al., 2012).

PCS in Marchigiana and Romagnola is associated with stillbirth and is characterised by facial deformities, enlarged fluid-filled abdomen, and hepatic fibrosis. It is caused by the KDM2B:p.Asp835Asn missense variant (Testoni et al., 2012; Murgiano et al., 2020).

CC in Romagnola is characterised by an immature bilateral nuclear cataract. It is caused by a deletion affecting the coding region of *NID1* (Murgiano et al., 2014).

HFM is a recently congenital malformation reported in a single Romagnola calf, characterised by microtia, asymmetry of the face and deafness. It is caused by the LAMB1:p.Arg668Cys missense variant (Jacinto et al., 2022).

IF in Chianina is a severe congenital, not compatible with life genodermatosis characterised by a skin completely covered with large horny plates separated by deep fissures and resembling a 'leather cuirass'. It is caused by the ABCA12:p.His1935Arg missense variant (Charlier et al., 2008).

IC in Chianina is a congenital genodermatose characterised by inelastic leather cuirass-like skin associated to generalised hypotrichosis and local alopecia, accompanied by urolithiasis, retarded growth and loss in leather quality. It is caused by an insertion in *FA2H* causing a frameshift (Jacinto et al., 2021).

Currently, the PMT1-causing variant in Chianina and Romagnola, the PCS-causing variant in Romagnola, and the IF-causing variant in Chianina are routinely included in the breeding programme of the Italian Association of Italian Beef Cattle Breeders.

The aim of this study was to characterise the allelic frequency and the carrier prevalence for the seven known harmful alleles in Italian Chianina, Marchigiana and Romagnola cattle.

## Material and methods

In total, 3331 Chianina, 2812 Marchigiana and 1680 Romagnola male animals were included in the study. The study involved semen samples from artificial insemination (**AI**) sires top listed in the annual catalogue, as well as blood samples from young males shortlisted for admission to performance testing at the testing station. Genomic DNA was isolated from these samples using standard methods. Three variants (ATP2A1:p.Arg164His; KDM2B:p.Asp835Asn; ABCA12:p.His1935Arg) were genotyped either by Sanger sequencing of PCR products as described before (Charlier et al., 2008; Drögemüller et al., 2008; Testoni et al., 2012) or by extraction of available SNP array genotyping data from routine genomic selection. Further four variants (ATP2A1:p.Glu211Val; *NID1* deletion, LAMB1:p.Arg668Cys; *FA2H* insertion) were genotyped via PCR-based methods (Murgiano et al., 2012 and 2014; Jacinto et al., 2021 and 2022). Table 1 summarises the information on the total number of genotyped males per disorder, associated variant and breed.

## Results

Using the total of almost eight thousand genotyping data available or obtained for this study, the allele frequencies (**AFs**) of the seven known causative variants were determined, and an assessment of the prevalence of heterozygous carriers within populations and between breeds was performed. Among the three breeds of beef cattle kept in Italy, carriers for PMT1 were found in each breed, carriers for PCS in two breeds, while carriers of the other five variants were evidenced only in the population in which

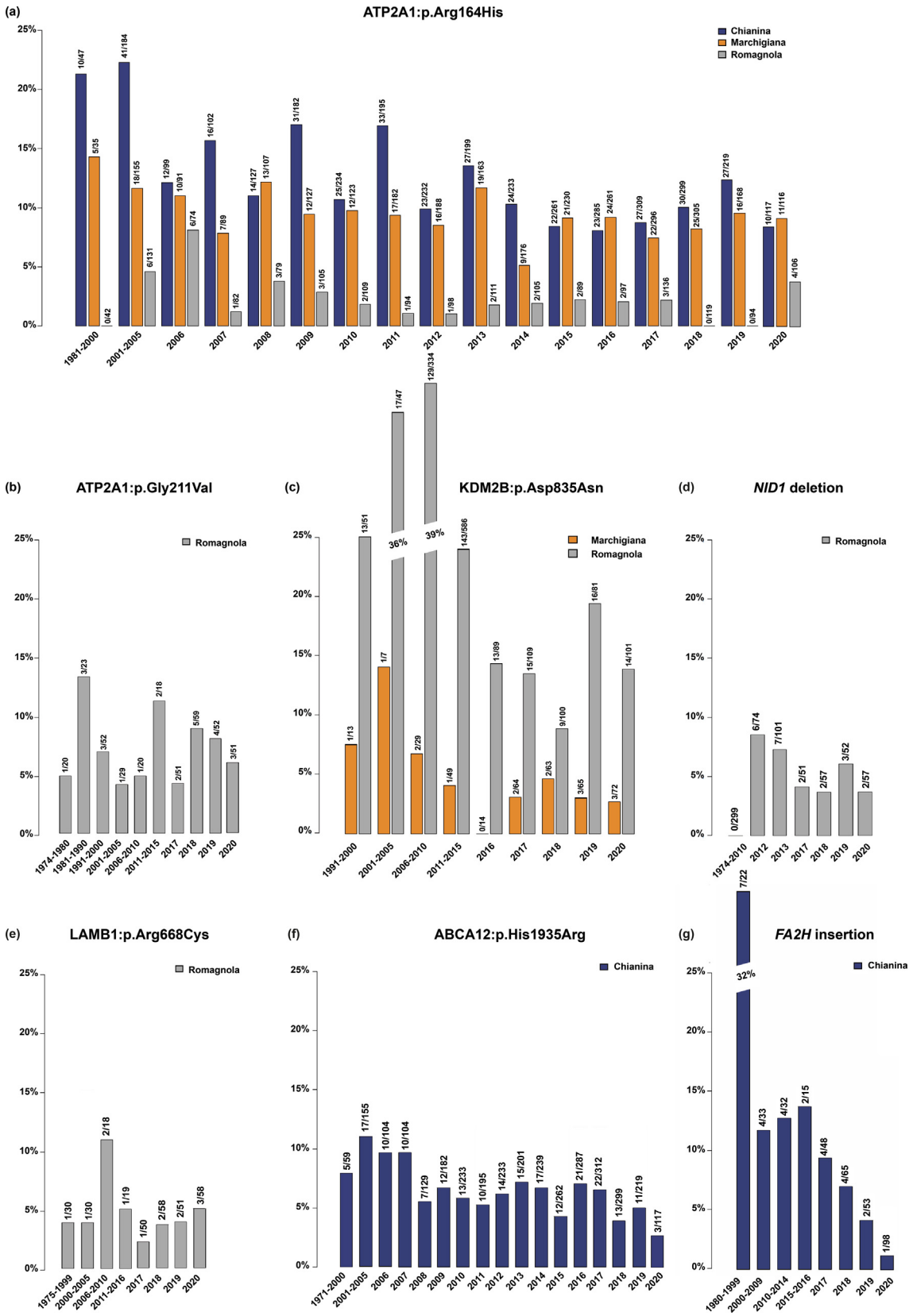


**Fig. 1.** Prevalence of heterozygous carriers for six recessively inherited disorders in Chianina, Marchigiana and Romagnola cattle. (a) PMT1, (b) PMT2, (c) PCS, (d) CC, (e) HFM, (f) IF, (g) IC. Note that the presented information has been based on the number of young bulls eligible for admission to the performance test and sorted by year of birth. PMT1 = pseudomyotonia congenita ATP2A1 p.Arg164His; PMT2 = pseudomyotonia congenita ATP2A1 p.Gly211Val; PCS = paunch calf syndrome; CC = congenital bilateral cataract; HFM = hemifacial microsomia; IF = ichthyosis fetalis; IC = ichthyosis congenital.

they were previously described (Table 1; Fig. 1). In summary, five of the seven variants occurred in the Romagnola, whereas three defect alleles segregated in the Chianina and only two in the Marchigana.

The derived AF and prevalence of PMT1-carriers varied between breeds, being lowest in the Romagnola and highest in the Chianina. In the Marchigiana, the AF in the cohorts of the last birth years was partly even higher than in the Chianina, where a decreasing tendency could be seen after the introduction of the genetic test more than 10 years ago (Fig. 1a). In the Romagnola, on the other hand, the derived AF and the prevalence of PMT2 carriers were additionally relatively high and in the sum of PMT1 and PMT2, the figures for this breed were in the range of PMT1 in the Chianina (Fig. 1a + b).

The PCS allele was by far the most frequent in Romagnola and also occurred with low frequency in Marchigiana while carriers of PCS were not detected in Chianina (Table 1). The derived AF and the prevalence of PCS-carriers in Romagnola were 11.7 and 23.4%, respectively, whereas in Marchigiana, the figures were almost ten times lower (Table 1; Fig. 1c). The PCS allele was originally even more common in the Romagnola than what was found in more recent time, evidencing that a clear decline has occurred since the introduction of the genetic test in 2011. Nevertheless, the AF fluctuated in the last years (Fig. 1c).

The other four deleterious alleles, causing CC, HFM, IF and IC, each occurred only in the breed in which they were previously detected, moreover with lower frequency (Table 1). The variants of CC and HFM occurred only in Romagnola, and the variants for IF and IC occurred only in Chianina. With respect to CC, genotyping revealed individual CC-carriers only in Romagnola born after 2010, and the frequency of the derived allele was comparatively lowest at 1.6% (Fig. 1d). The HFM-causing allele was comparatively rare (1.9%) (Table 1), but only half of the animals have been examined so far, as the gene variant was discovered only recently. The HFM-carriers identified are distributed relatively evenly across all birth cohorts examined (Fig. 1e). In Chianina, the derived allele frequency and the prevalence of IF-carriers were 3.2 and 6.4%, respectively. The number of carriers for the overall longest known variant has decreased slightly over the period considered, but heterozygous males were still detected annually (Fig. 1e). The frequency of the recently identified variant causing IC and the corresponding prevalence of carriers were calculated comparatively higher at 3.8 and 7.7%, respectively. Remarkably, the overall occurrence of heterozygous IC-carriers is decreasing over time, but so far fewer animals have been studied compared to all other variants (Fig. 1g).

## Discussion

The identification of the molecular cause of recessively inherited disorders allows screening of populations to identify heterozygous carriers in the population of active and potential future breeding animals and thus to create the basis for a sustainable selection aimed at producing as few affected calves as possible. In this study, we comprehensively describe the prevalence for six known recessive disorders in Chianina, Marchigiana and Romagnola.

Most of the gene variants studied here probably occurred far away in the past, since on the one hand, carriers can be found in more than 40 years and on the other hand, the alleles partly occur across breeds. Since no PMT1-carriers were found in Romagnola born before 2001, one can speculate about a possible introgression of the allele that was very common in Chianina and Marchigiana at that time. The absence of CC-carriers in older animals indicates a recent origin of the variant in the Romagnola population. Since isolated HFM-carriers have also been found in older animals, it can be assumed that the *LAMB1* mutation arose comparatively earlier.

Phylogenetic studies suggest that Chianina, Marchigiana and Romagnola have a close common ancestor, with Chianina and Marchigiana being evolutionarily closer and related to Romagnola (Mastrangelo et al., 2018). Therefore, this might also represent an explanation for the presence of PMT1-carriers in all the three studied breeds, as well as for the PCS allele in Marchigiana and Romagnola. Indeed, considering these facts, we speculate that PMT1 represents the oldest mutation, followed by PCS.

In this study, we evidence for the first time the presence of PMT1-carriers in Marchigiana, which opens the basis to improve the fitness of the breed by selecting against this allele. In the particular case of PCS in Romagnola and PMT1 in Chianina, the results showed that the strategy of preventing heterozygotes to be admitted to the performance testing needs more time. A possible reason why deleterious alleles keep segregating at a considerable allele frequency is the so-called balancing selection (Fasquelle et al., 2009), where linked variants have desirable effect on production traits and are therefore under positive selection. In this regard, there is a need for further research, especially for Romagnola.

Even if the other defect alleles such as PMT2, CC or HFM, which often only occur in one breed, are comparatively less common, caution is indicated. Therefore, they should be also considered in future selection programmes. Indeed, the use of heterozygous popular AI sires could increase the spread of deleterious alleles in populations. The actual number of affected animals of these disorders in Italy is not known, but since carriers have been found, sporadic cases may occur more or less often, depending on the different AF.

In conclusion, this study validates the concerns regarding the impact of recessively inherited disorders in the Italian beef cattle considered in the study. Moreover, it evidences that the PMT1 and PCS alleles are not restricted to the population in which they were discovered. Genotyping for all seven known harmful alleles, in both male and female cattle, is recommended to prevent risk mating between carriers, in particular to avoid affected offspring in the interest of animal welfare.

## Ethics approval

This study did not require official or institutional ethical approval as it was not experimental, but rather part of routine genotyping tests.

## Data and model availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions. None of the data were deposited in an official repository.

## Authors ORCIDs

**J.G.P. Jacinto:** https://orcid.org/0000-0002-6438-7975.
**A. Gentile:** https://orcid.org/0000-0002-6091-8978.
**C. Drögemüller:** https://orcid.org/0000-0001-9773-522X.

## Author' contributions

**JGPJ:** Conceptualisation, Investigation, Methodology, Visualisation, Writing – Original Draft, Writing – Review and Editing**;** **FS:** Resources, Writing – Review and Editing; **AQ:** Resources, Writing – Review and Editing; **AG:** Resources, Writing – Review and Editing; **CD:** Conceptualisation, Supervision, Writing – Original Draft, Writing – Review and Editing.

## Declaration of interest

None.

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