ABSTRACT: RYR1 is a calcium release bomb that is located in the sarcoplasmic reticulum. In domestic animals, several disorders of skeletal muscle are caused by numerous mutations, and they can alter meat quality, like R615C in pork. Protein sequences and missense mutations reported in bovine and porcine RYR1 were used in order to obtain the effect of these substitutions on normal function of the protein. Assessed physic-chemistry traits were electrostatic potential, isoelectric point, and surface structure in two wild models (bovine and porcine) and nine mutated alleles. Several bovine alleles (827E, mainly) have an important alteration of electrostatic potential. This trait is also altered in the 615C allele in pork. Alteration of electrostatic potential can modify interaction intra y/o inter-molecule.

Key words: model; skeletal muscle; electrostatic potential; R615C; porcine.

INTRODUCTION

In skeletal muscle, calcium release is mediated by a huge channel (=2.2 MDa) (Lobo and Van Petegem (2009); Serysheva et al. (2005)), Ryanodine receptor 1 (RYR1) (Ludtke et al. (2005)). This bomb is a heterotetrameric complex that is located in the sarcoplasmic reticulum (SR) (Serysheva et al. (2008)) membrane. Each subunit of RYR1 is composed of 5037 (or 5032) amino acid residues (Serysheva et al. (2005)). Muscle contraction requires elevation of calcium in the myoplasm, and uptake of calcium by the SR enables muscle to relax (Marks et al. (1989)). Neuronal-induced depolarization of the plasma membrane is the stimulus for calcium release (Wagenknecht and Radermacher (1997)). Ryanodine receptors are interesting bombs because they play a central role, both in a structural sense and a functional sense, in this signal-transduction process, which is known as excitation-contraction (EC) coupling (Wagenknecht and Radermacher (1997)). Activation of RYR1 occurs through interaction with the Dihydropyridine Receptor (DHPR), located in the transverse tubular membrane, directly opposite to RYR1 in the SR membrane (Serysheva et al. (2005)). DHPR is an L-type calcium channel of exterior membranes, which acts as the voltage sensor of excitation-contraction coupling (Felder et al. (2002); Marks et al. (1991); Hwang et al. (2012); Marks et al. (1989)).

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder of skeletal muscle that segregates with >60 mutations within the MHS-1 locus (RYR1) (Yang et al. (2003)). In pork, one of these mutations is R615C, this missense mutation have the largest influence on meat quality, and stress susceptibility (Lahucky et al. (1997); Otto et al. (2007)). The object of this study was to examine the effect of reported missense mutations of bovine RYR1 gene through computational tools.

MATERIALS AND METHODS

Sequences and mutations

Sequences NP_001193706.1 (bovine wild) and NP_001001534.1 (porcine wild) were used. The missense mutation used, are shown in table 1, and were located manually in each sequence. Additionally, the porcine mutation R615C (n allele) was included in this analysis because it has an important phenotypic effect (Ta et al. (2007)).

Protein models: Modeling by homology was used from amino acid 12 to 532 with SwissModel software (Bordoli et al. (2009); Arnold et al. (2006); Guex and Peitsch (1997); Xiang (2006)). The remaining amino acids (4508) were split into segments of 120 residues and modeled with I-TASSER software (Zhang (2008); Roy et al. (2010)). These segments were spliced in 9 models of 500 amino acids. Deep Viewer was used for visualization (Guex and Peitsch (1997); www.expasy.org/spdbv). Additionally, energetic minimization was made through Gromos96. Wild model of bovine (533-1000 amino acids) was used to model wild and 615C allele of swine. The missense mutation used are all of mutated sequences of bovine (alleles of table 1) through Swiss Model.

RESULTS AND DISCUSSION

Electrostatic potential was the most altered trait by these mutations at in-silico level. Figure 1 shows the electrostatic potential of wild porcine model and its 615C allele (a. and b., respectively). Bovine polymorphisms C762W, K827E and E832G can also alter this trait in relation to bovine wild model.

Meat from non-carrier pigs of the n allele possess darker muscle color, higher muscle structure scores, more marbling and more desirable retail appearance (Jeremiah et al. (1999)). On the other hand, carriers of this allele have the higher loss of water from meat and this fact has high economic importance because this meat has a substantial reduction in weight (Otto et al. (2007)); but, there is an unfavorable antagonism between meat quality and carcass traits in this locus (Otto et al. (2007)). In Cattle, there is not a reported mutation with a similar phenotypic effect to porcine R615C allele. Although, in this paper, several alleles (827E, mainly) have an important alteration of electrostatic potential, in the same way that 615C allele does it. R615C shows heightened sensitivity to activation and altered regulation by physiological cations that result in an uncontrolled or spontaneous Ca2+ release from the SR (Ta and Pessah (2007)). In this way, Ta and Pessah (2007) and Yang et al. (2003) reported that R615C shows more pronounced activation by Ca2+, and is less sensitive to channel inhibition by Ca2+ and Mg2+, compared to wild allele. Additionally, myotubes at rest of carrier pigs can not be fully inactivated at Ca2+ typical level, and this
Alters electrostatic potential of this segment (533-1000) of RYR1 protein can modify interaction inter-
molecules, for example with its activator DHPR (Yang et al. (2003)) or with another regulator. Serysheva et al. 
(2005) highlighted that this bomb combines large size with 
complex allosteric modulation of channel opening, thus this 
protein has a great conformational variability, with a 
mixture of different functional states; in that manner, 
numerous proteins are present at these junctions, some of 
which interact directly with RYR1 (Wagenknecht and 
Radermacher (1997)). RYR1 can be modulated by a variety 
of physiological ligands including Ca$^{2+}$, Mg$^{2+}$, Calmodulin, 
FKBP12, and ATP (Serysheva et al. (2005); Sencer et al. 
(2001); Serysheva et al. (2008); Xiong et al. (2002); Cornea 
et al. (2010)). Another possible mechanism can be through 
interaction intra-molecule, because RYR1 have several 
regions with biological functionality; for example, its N-
terminal end (it is needed in order to allow normal EC 
coupling-Perez et al. (2003)), EF hands between amino 
acid positions 4081 and 4092 (EF1) and 4116 and 4127 
(EF2) (Fessenden et al. (2004)) and an oxidoreductase 
domain in the N-terminal region (Baker et al. (2002)).

Table 1. Wild sequences and mutations of RYR1 and their 
effect in isoelectric point

<table>
<thead>
<tr>
<th>Accession number</th>
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<tr>
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<td>C</td>
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</table>

Figure 1. Electrostatic potential and alterations generated by missense mutations.
Model 2 (533-1000 amino acids) a. wild porcine, b. 615C porcine allele, c. bovine wild, d. 762W bovine allele, e. 827E 
bovine allele, and f. 832G bovine allele.
Although, the location of the \( \text{Ca}^{2+} \) activating sites on RYR1 bomb remains a mystery (Hamilton (2005)).

Molecular mechanisms by which \( \text{Ca}^{2+} \) and other activators can open this channel and elucidation of the effects of mutations on this process are perhaps the greatest challenges ahead in the EC coupling (Hamilton (2005)). R615C mutation markedly enhanced the luminal \( \text{Ca}^{2+} \) activation of RYR1 (Jiang et al. (2008)), and electrostatic potential can explain the effect of this mutation, affecting the intrinsic properties of RYR1 channel and the propensity for spontaneous \( \text{Ca}^{2+} \) release during store \( \text{Ca}^{2+} \) overload. This process is referred to as store overload-induced \( \text{Ca}^{2+} \) release (SOICR) by Jiang et al. (2008).

**CONCLUSION**

In Cattle, there is not any reported mutation with a similar phenotypic effect to porcine R615C allele; but in this analysis of bovine polymorphisms, C762W, K827E and E832G have an important alteration of electrostatic potential of a segment of RYR1 protein. This molecular trait is also modified by the porcine 615C allele (with respect to wild type), and this is the first report of a biological explanation (in-silico) of its phenotypic effect.

**LITERATURE CITED**

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