POLYMORPHISMS IN PLASMA MEMBRANE CALCIUM-TRANSPORTING ATPASE 1 (*ATP2B1*) GENE IN HENS

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Abstract: Bone fragility in caged laying hens is a severe welfare problem. This fragility has been attributed to osteoporosis, which etiology is multifactorial in birds, as well as in humans, with genetic, environmental, and nutritional components. *Plasma membrane calcium-transporting ATPase* 1 gene (*ATP2B1*) is in hens located on chromosome 1, region 43 273 706 – 43 305 815 bp. This gene has 21 exons, three of them were genotyped. In this study we genotyped 110 hens of ISA BROWN hybrids. Genotypes of *ATP2B1* gene were determined using PCR-RFLP in exons 10 and 12. Genotypes of two SNPs in exon 8 were determined using sequencing. In our group of animals, only allele without deletion in exon 10 and only allele *A* in exon 12 was found. In exon 8 subsequent genotypes were detected: in C61T locus *CC* and *TT;* in C80T locus *CC, CT* and *TT.*

Key Words: ATP2B1, gene, sequencing, exon

INTRODUCTION

Bone fragility is a general welfare problem in caged laying hens, with fracture incidences in commercial flocks over 30% of all hens during their life. This fragility has been attributed to osteoporosis, which etiology is multifactorial in birds, as in humans, with genetic, environmental, and nutritional components (Fleming et al. 2000).

The Plasma membrane calcium-ATPases (PMCAs or Ca^{2+}ATPase) are a group of more than 30 isomers that use the energy stored in ATP to extrude Ca^{2+} out of the cell against the electrochemical gradient (Davis et al. 1987; Wasserman et al. 1992, Bouillon et al. 2003, Stokes and Green 2003, Belkacemi et al. 2005, Hoenderop et al. 2005, Nijenhuis et al. 2005). Briefly, the PMCA1b is the predominant isomer expressed in the mammalian intestine, kidney and placenta (Howard et al. 1993, Nijenhuis et al. 2005) and the chicken intestine (Melancon and DeLuca 1970, Strittmatter 1972, Davis et al. 1987) and kidney (Qin, Klandorf 1993). In the intestine, kidney and placenta the PMCAs are located on the basolateral membrane of the epithelial cell toward which Ca^{2+} is transported (Borke et al. 1989a, 1989b, 1990). The intestinal expression at the transcriptional level is modulated by vitamin D (reviewed in Zelinski et al. 1991, Wasserman et al. 1992) and also by a variety of factors that affect vitamin D metabolism (Wasserman et al. 1992, Cai et al. 1993, Armbrecht et al. 1994, Zhu et al. 1998).

Plasma membrane calcium-transporting ATPase 1 gene (*ATP2B1*) in hens is located on chromosome 1, region 43 273 706–43 305 815 bp and has 21 exons (Ensembl 2015).

MATERIAL AND METHODS

In this study 110 animals of ISA Brown hybrids in the average age of 15 week were used. Blood sampling was provide from *vena brachialis* and blood stabilized with heparin. Isolation of DNA was carried out from 100 µl blood. For isolation of DNA a commercially available DNA Lego kit (Top-Bio, Prague, Czech Republic) was used. The isolation proceeded according to the manufacturer's protocol.

Ensemble database was used to *in silico* analysis with the aim to search SNP in this gene, preferentially causing amino acid changes.

Polymorphisms of *ATP2B1* gene were studied in three exons: 8, 10 and 12. The PCR reactions was performed at volume 15 µl, with 10 pmoles of each primer (IDT Inc., Coralville, USA), 2 x PPP ™ MasterMix (Top-Bio, Prague, Czech Republic), ultrapure H_2O (Top-Bio, Prague, Czech Republic) and 50 ng DNA. The primers were designed using the OLIGO software v4.0 (Molecular Biology Insights, Inc., Colorado Springs, CO, USA) according to sequences from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). The primer sequences were: exon 8 forward: 5'-GAGAAATGTTTGCCCCTTGAC-3' and reverse: 5'-CCAAAGATGCCAGTGTCACAC-3'; exon 10 forward: 5'-AAAACTGAATGTGCCTTGCTG-3' and reverse: 5'-CAAGGGTAAAGGACTG TTGCAC-3'; exon 12 forward: 5'-TTACATGTAGGTACCCGATGCA-3' and reverse: 5'-GCCTTTACAGAACAGCTGATCC-3'. Temperature profile of PCR reactions were 95/3min; (95°C/20s; 59°C /30s; 72°C /60s) 35x; 72°C /7min; 7°C /∞.

Results from PCR reaction were tested by electrophoresis on 3% agarose gel and visualized by EtBr. Fragment size was compared with a weight marker 50 bp DNA Ladder (M50) and 100 bp DNA Ladder (M100) (Thermo Fisher Scientific Inc., Waltham, USA).

Most RFLP reactions were carried out in a volume of 15 µl containing 10x Buffer for restriction endonuclease (Thermo Fisher Scientific Inc., Waltham, USA), restriction enzyme *Alw*44I for exon 10 or *Bse*NI for exon 12 (Thermo Fisher Scientific Inc., Waltham, USA), PCR product, and ultrapure H_2O. Incubation of the reaction mixture was carried out at 37°C. After the incubation period, the samples were immediately analysed on 3% electrophoretic gel for genotype determination.

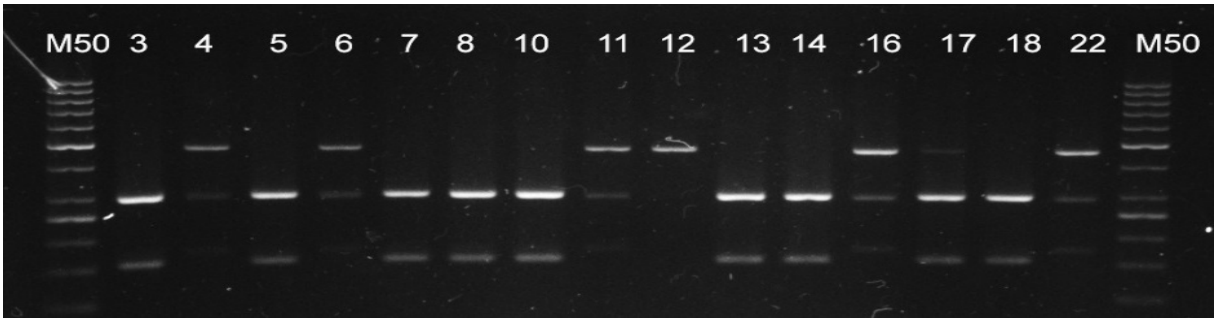
Exon 8 was sequenced according to the producer's protocol BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). It was using genetic analyser ABI PRISM 3500 (Applied Biosystems, Foster City, CA, USA) for sequencing.

RESULTS AND DISCUSSION

In this work we tested C61T and C80T substitutions in the exon 8, T128 deletion in exon 10 and A55C substitution in exon 12 of *ATP2B1* gene (Ensembl 2015).

We analysed two SNP in exon 8 (C61T, C80T); mutation C80T that does not cause an amino acid change. The determination of genotypes was done using sequencing, because reading genotypes from the electrophoretic gel was not possible due to poor separation of fragments (Figure 1). The PCR products in exon 8 had a size of 464 bp. The frequency of allele *C* is 0.75 and allele *T* is 0.25 in C61T locus. Frequency of genotype *CC* and *TT* in C61T locus is 0.75 and 0.25, respectively. Frequency of allele *C* and *T* in C80T locus is 0.83 and 0.17, respectively. Frequency of genotype *CC, CT and TT* in C80T locus is 0.75, 0.17 and 0.08, respectively.

Figure 1 Detection of genotypes in exon 8 using BstNI



The PCR products of exon 10 had a size of 345 bp or 344 bp in case of deletion at nucleotide T. Detection of deletion was made using the restriction enzyme *Alw*44I. In our group of animals only allele without deletion was found, all animals were monomorphic.

The PCR products of exon 12 had a size of 233 bp. Detection of *A/C* polymorphism was made using the restriction enzyme *Bse*NI. *A* allele is characterised by digestion of the PCR fragment to sizes 163 and 69 bp and *C* allele is not digested. Among the group of animals only *A* allele was found.

Ensembl (2015) describes polymorphisms in exon 10 and 12, but in our group of animals these loci were monomorphic.

CONCLUSION

The aim of this work was to determine variability in the exons 8, 10 and 12 of *ATP2B1* gene in hens. In the studied group of animals only allele without deletion in exon 10 and only allele *A* in exon 12 was found. Exon 8 was polymorphic and we found followed genotypes in C61T locus*: CC* and *TT,* in C80T: *CC, CT* and *TT.* Verification of presence of polymorphism enables performing of subsequent association analysis.

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