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Evaluation of the *RYR1* gene genetic diversity in the *Latvian White* pig breed

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Aim: The ryanodine receptor 1 (RYR1) is a calcium ion channel in the sarcoplasmic reticulum of skeletal muscle. Multiple polymorphic loci have been identified in the *RYR1* gene in human and animals and some of them are associated with certain phenotypes. However, there are still few data on the *RYR1* genetic variability in pig and only the missense mutation Arg615Cys, associated with the malignant hyperthermia, porcine stress syndrome and meat quality, has been studied in several commercial and local breeds. The aim of the current study was to genotype the rs344435545 (C1972T, Arg615Cys), rs196953058 (T8434C, Phe2769Leu) and rs323041392 (G12484A, Asp4119Asn) in the Latvian local pig breed *Latvian White* and to evaluate the eventual functionality of amino acid substitutions. **Methods:** Loci were genotyped by the restriction fragment length polymorphism technology in the collection of 8 samples of original *Latvian White* collected in 2006 and 103 samples of *Latvian White* collected in three Latvian geographically distant private farms in 2015. SIFT online tool was applied to evaluate a potential effect of the amino acid substitutions on protein functions. **Results:** The loci rs344435545 and rs196953058 were found to be monomorphic in both collections. On the contrary, the rs323041392 showed a high level of polymorphism in the original *Latvian White* with GG/GA/AA genotype correlation equal to 3/4/1 and the absence of polymorphism in 2015 collection. From the studied loci only the rs344435545 was identified as possessing potential to change functions of the protein. **Conclusions:** The unfavourable rs344435545 T allele having functional effect on the protein function, appears not to have been introduced in *Latvian White* pig breed. Full loss of the rs323041392 variability in *Latvian White* happened in nine years of private farming, could be a message to Latvian and other European livestock industry that the breeding intensification may decrease the genetic diversity, specific features performance and adaptability to the environmental challenges in local breeds of small populations.

Keywords: RYR1, polymorphism, genetic diversity, pig

Introduction

RYR1 encodes the ryanodine receptor 1, which is a calcium ion channel in the sarcoplasmic reticulum of skeletal muscle. In humans multiple single nucleotide polymorphisms (SNPs) in the coding gene region (cSNPs) are associated with the malignant hyperthermia susceptibility [1], central core disease [2], and other myopathies [3]. Remarkably, that a homologous missense mutations in human (Arg614Cys) and pig (Arg615Cys) originated from homologous C>T cSNPs (rs118192172 and rs344435545 for human and pig respectively) lead to the same malignant hyperthermia syndrome. Besides malignant hyperthermia the rs344435545T allele is associated in pig with the porcine stress syndrome (PSS, halothane susceptibility) and pale, soft and exudative meat (PSE) syndrome [4]. It is suggested,

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however, that heterozygote carriers in some breeds may have some adaptive advantages and better parameters for the economically important meat traits [5].

Besides the rs344435545 up today about 2000 SNPs and 150 cSNPs have been reported for the pig *RYR1* gene (http://www.ncbi.nlm.nih.gov/snp). However, there are still no data on their genetic diversity and possible association with phenotype.

The aim of the current study was to genotype the rs344435545 (C1912T, Arg615Cys), rs196953058 (T8434C, Phe2769Leu) and rs323041392 (G12484A, Asp4119Asn) in Latvian local pig breed *Latvian White* and to evaluate *in silico* the potential effect of amino acid substitution on the protein function.

Materials and methods

Sample collections include the Primary (PR) collection of 8 samples of original *Latvian White* randomly collected from Latvia University of Agriculture (LLU) herd in 2006 within Latvian Ministry of Agriculture supported project and Representative (RP) collection of 103 samples collected in 2015 in three geographically distant private farms in frame of the "**AgroBioRes**" Latvian National Research Program (VPP) 2014-2017. DNA was isolated from 500 µl of blood of each animal by using a K0512 Genomic DNA Purification Kit (Thermo Scientific). Sequence information for swine (*Sus scrofa*) *RYR1* gene available in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/347616829?report=fasta&from=1714228&to=1862032) was used for the genomic region reconstruction and PCR primer design. Figure 1 provides a detailed information on the conserved domain RYR1 protein architecture and location of the genotyped polymorphic loci.

All three polymorphisms were genotyped by a restriction fragment length polymorphism (RFLP) technology. Primer sequences and other PCR and RFLP related information are given in Table 1.

PCR was performed using DreamTaq polymerase (Thermo Scientific) with following DNA amplification parameters: 94 °C for 5 min; then 40 cycles of 94 °C for 30 s, appropriate annealing temperature (60.2-65.6 ˚C) for 30 s, 72 °C for 45 s and 72 °C for 5 min. DNA digestion with restriction enzymes was performed according to the producer protocol (Thermo Scientific). The PCR and restriction products were analysed by electrophoresis in 1–3 % agarose gel. Genotyping was performed in duplicate for each locus of each animal. The genotyping results were verified by sequencing in both directions (ABI Prism3100 Genetic Analyzer) of randomly chosen amplicons.

The prediction of potential effect of amino acid substitutions on the protein function was performed using online available tool SIFT (Separate Intolerant from Tolerant, http://sift.bii.a-star.edu.sg/) following the recommendations of developers [6, 7]. The pig RYR1 sequence with the accession P16960 used as a query was compared with the related sequences found by SIFT in SwissProt database. The sequence

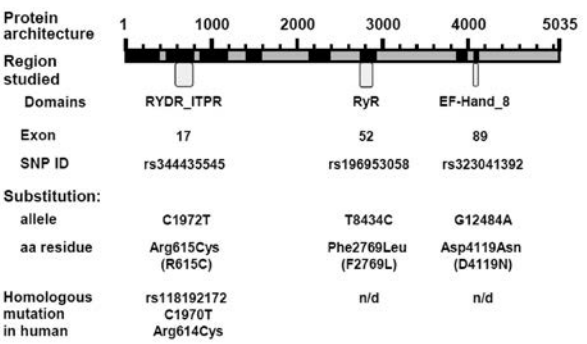

Fig. 1. Pig (*Sus scrofa*) RYR1 protein sequence scheme given along with information on the mutations studied. Upper line illustrates the polypeptide chain architecture where the conserved domains are indicated in black. The conserved domains and exons encompassing the studied cSNPs are indicated below by abbreviation and number for the domains and exons respectively. SNPs' description include the SNP ID number and nucleotide and amino acid residue change in mRNA (NM_001001534.1) and polypeptide (NP_001001534.1) respectively given according to SNP database at NCBI (http://www.ncbi.nlm.nih.gov/snp). Information on the homologous mutation in human is given in the bottom of the picture. Abbreviations: RYDR_ITPR – the RIH extracellular domain found in both the ryanodine and the inositol-1,4,5- trisphosphate receptors types of calcium channels; RYR – the ryanodine receptor specific conserved domain of unknown function; C – EF-hand_8 – the conserved domain of the cl08302 superfamily, contains calcium sensors and calcium signal modulators; n/d – no data.

Table 1. **Details of the genotyping technology**

SNP ID	Primer sequences	Enzyme / restriction site	Fragment size amplified / restricted (bp)
rs344435545*	F-5'-CCTGGGACATCATCCTTCTG-3' R-5'-GGTGGTGGAGGGTTCTAAGC-3'	*Hha*I / 5'-G[C]G^C-3'	282 / 150+132
		*Alw*21I / 5'-G[W]GCW^C-3'	282 / 152+130
rs196953058#	F-5'-ATGTTTCAGGGGTGGAAGGC-3' R-5'-AGGGAAATGTGGGGCCCTTG-3'	*Apa*I / 5'-GGGCC^[C]-3'	#1246 / 209+25+12 #2246 / 107+102+25+12
rs323041392	F-5'-GCCATGGACAGCCAGAAGCA-3' R-5'-CCCAGGTAGGGCCGGAAGTA-3'	*Bcl*I / 5'-T^GATC[A]-3'	251 / 171+80

* locus was genotyped according to Silveira *et al.* (2011) [8] simultaneously by two restriction enzymes: *Hha*I cuts the sequence having major favourable allele C and *Alw*21I cuts the sequence encompassing unfavourable allele T;

*Apa*I cuts two and three restriction sites in the sequence encompassing the rs196953058 T (#1) and C (#2) alleles respectively. A polymorphic nucleotide is boxed in the restriction site description.

median conservation was set to 3.00 prior the analysis [6]. For every amino acid in each position in the alignment, SIFT calculates normalized probabilities for all possible amino acid substitutions. The substitution is predicted as deleterious if normalized values are less than cut-off (0.05) [7].

The research was approved by the LLU review board.

Results and Discussion

All polymorphic loci studied are cSNPs located in the *RYR1* gene evolutionary conserved domains (Figure 1, 2). Figure 2 illustrates a homology between the related sequences of human and pig proteins and a high level of homology in the positions and motifs of amino acid substitutions.

The conserved domains are thought to be responsible for binding different modulators of the protein function [9]. Therefore, amino acid substitution in the RYR1 domains may significantly change the protein target affinity and response to the regulators. We performed an *in silico* analysis using SIFT online tool to detect, whether an amino acid substitutions in the loci studied may affect the RYR1 function. We found out that the R615C substitution potentially possessed a damaging influence on the RYR1 function (SIFT score 0.01) while the F2769L and D4119N substitutions appear to be neutral (SIFT scores 1.00 and 0.24, respectively).

Our findings on the R615C functionality is in good correlation with well-known data on the mutation phenotypic effect in both human [1, 10] and pig [8]. In

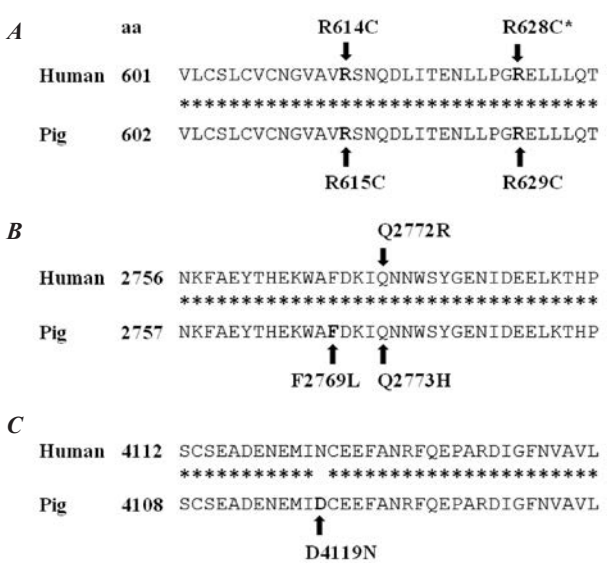

Fig. 2. Partial alignment of the human (P21817.3/GI:108935904) and pig (P16960.2/GI:1173335) RYR1 protein sequences. Panels A, B and C illustrate alignment of the regions encompassing the rs344435545, rs196953058 and rs323041392 of the pig protein respectively. Arrows indicate a position of mentioned and neighbouring amino acid substitutions in the human (upper line) and pig (lower line) protein, motif description is given above the corresponding arrows. *there are two variants of amino acid substitution at the 628: besides indicated R628C substitution the R628H also has been reported. Abbreviations: aa – amino acid.

contrast to human where the mutation is dominant [11], the malignant hyperthermia, PSS and PSE syndromes in pig are associated with a homozygous genotype [12] and the adaptive advantages and better parameters for economically important meat traits were reported for the heterozygous animals [5, 8].

Figure 3 illustrates genotyping results.

The locus rs344435545 was found to be monomorphic in both PR and RP collections. Despite the fact, that a related unfavourable phenotype (malignant hyperthermia, PSS) had not been reported for *Latvian White* until now, the full absence of site polymorphism in this breed was not expected before our study. The rs344435545 minor (unfavourable) T allele had been detected in the pigs of *Yorkshire* breed [13] used in *Latvian White* initial selection (http://www.ccc.lv/lv/selekcija/latvijas_balta.htm) and often used in current breeding programs (http://www.ccc.lv/lv/selekcija/latvijas_balta.htm). Heterozygote carriers of the rs344435545 T allele of *Yorkshire* breed could be supported by breeding programs as having adaptive advantages and better meat quality parameters [5]. However, this allele appears not to have been introduced in *Latvian White*. A permanent control using molecular markers is required to prevent introducing of unfavourable 1912T allele from *Yorkshire* pigs to *Latvian White* in the future breeding programs.

The locus rs196953058 was also observed as monomorphic in both PR and RP collections.

Instead, the rs323041392 showed in the PR a high level of polymorphism with the GG/GA/AA genotype correlation equal to 3/4/1. As the LLU herd (PR collection) had been under a strong selection control focusing on the *Latvian White* breed maintenance and conservation, polymorphism at the rs323041392 observed in the PR collection could be considered as a breed specific character. However, in the RP collection (animals from private farms) this locus was found to be monomorphic. So, full loss of the rs323041392 variability in *Latvian White* happened in nine years (from 2006 till 2015) of private farming. The loss of heterozygosity at the rs323041392 may signal to Latvian livestock industry that the

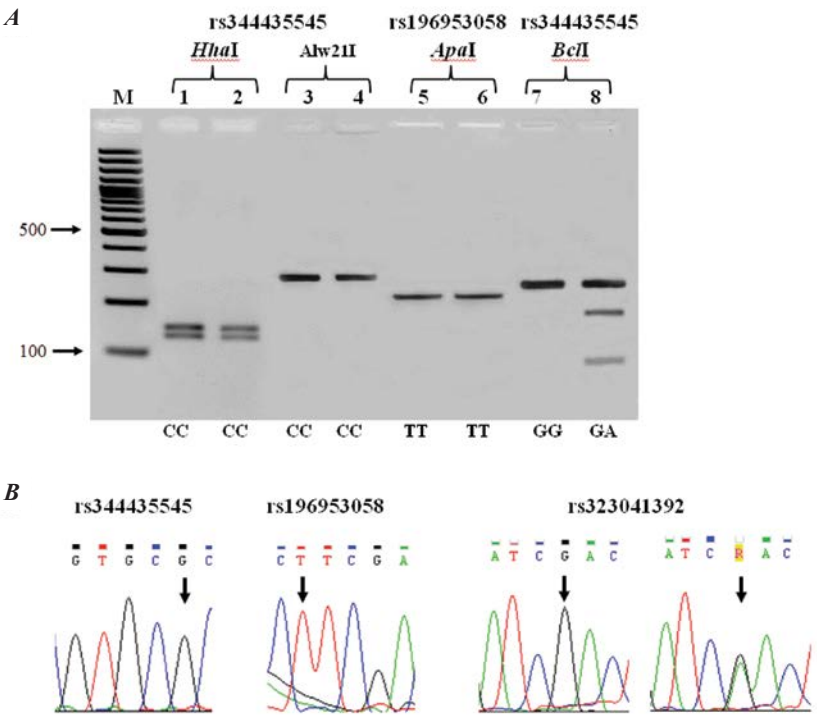

Fig. 3. Genotyping results given for two samples from PR collection. Panel A illustrates RFLP data; restriction enzymes and genotypes defined are indicated below and lower electrophoregrams respectively. Letter M indicates 100 bp DNA ladder (Thermo Scientific). Panel B provides examples of sequencing chromatograms: the rs344435545 and rs196953058 were found to be homozygous on the C and T alleles respectively, the rs323041392 was observed in the PR collection either as homozygote GG or as heterozygote GA.

breeding intensification and over-introgression of favourable alleles may lead to the loss of local breeds' specific features and decrease in the selective facilities and adaptability to the environmental challenges in the local breeds of small populations.

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Оценка генетического разнообразия генов RYR1 в латвийской белой породе свиней

К. Докане, Д. Йонкус, Т. Съякстe

Рецептор ріанодину 1 (RYR1) є кальцієвим іонним каналом саркоплазматичного ретикулуму скелетних м'язів. У гені *RYR1* людини і тварин були виявлені кілька поліморфних локусів, деякі них пов'язані з певними фенотипами. Однак даних про генетичну мінливість *RYR1* у свиней мало: для декількох комерційних і місцевих порід показана міссенс мутація Arg615Cys, пов'язана зі злоякісною гіпертермією, синдром свинячого стресу і якістю м'яса. **Мета.** Оцінити можливу функціональність амінокислотних замін у генотипах rs344435545 (C1972T, Arg615Cys), rs196953058 (T8434C, Phe2769Leu) і rs323041392 (G12484A, Asp4119Asn) місцевої латвійської білої породи свиней. **Методи.** Локус поліморфізму генотипували рестрикційними аналізом довжини фрагментів. Проаналізовано 8 зразків оригінальної латвійської білої породи свиней, зібрані в 2006 році і 103 зразків латвійської білої, відібрані в трьох латвійських географічно віддалених фермерських господарствах у 2015 році. Для оцінки потенційного ефекту амінокислотних замін на функції білка застосований онлайн-інструмент SIFT. **Результати.** Локуси rs344435545 і rs196953058 мономорфні в обох колекціях. При цьому rs323041392 показав високий рівень поліморфізму в оригінальній латиської білій породі з GG / GA / AA генотипом і кореляцією – 3/4/1 і відсутність поліморфізму в колекції 2015 році. З вивчених локусів, тільки rs344435545 має потенціал зміни функції білка. **Висновки.** Несприятливий на функцію білка аллель T в rs344435545, не присутній в латвійській білій породі свиней. За дев'ять років у фермерських господарствах у латвійській білій породі свиней сталася втрата мінливості rs323041392. Латиській і європейської тваринницькій галузі необхідно врахувати, що інтенсифікація розмноження може знизити генетичну різноманітність, конкретні робочі характеристики і адаптивність до екологічних проблем у місцевих порід у невеликих популяціях.

Ключові слова: RYR1, поліморфізм, генетична різноманітність, свині.

Оценка генетического разнообразия генов RYR1 в латвийской белой породе свиней

К. Докане, Д. Йонкус, Т. Съякстe

Рецептор рианодина 1 (RYR1) является кальциевым ионным каналом саркоплазматического ретикулума скелетных мышц. В гене *RYR1* человека и животных были обнаружены несколь-

ко полиморфных локусов, некоторые из которых связаны с определенными фенотипами. Однако данных о генетической изменчивости *RYR1* у свиней мало: миссенс мутация Arg615Cys, связанная с злокачественной гипертермией, синдром свиного стресса и качеством мяса показана для нескольких коммерческих и местных пород. **Цель.** Оценить возможную функциональность аминокислотных замен в генотипах rs344435545 (C1972T, Arg615Cys), rs196953058 (T8434C, Phe2769Leu) и rs323041392 (G12484A, Asp4119Asn) местной латвийской белой породы свиней. **Методы.** Локус полиморфизма генотипировали рестрикционным анализом длины фрагментов. Проанализированы 8 образцов оригинальной латвийской белой породы свиней, собранные в 2006 году и 103 образцов латвийской белой, отобранные в трех латвийских географически удаленных фермерских хозяйствах в 2015 году. Для оценки потенциального эффекта аминокислотных замен на функции белка применен онлайн-инструмент SIFT. **Результаты.** Локусы rs344435545 и rs196953058 мономорфны в обоих коллекциях. При этом rs323041392 показал высокий уровень полиморфизма в оригинальной латышской белой с GG / GA / AA генотипом корреляции, равном 3/4/1 и отсутствие полиморфизма в коллекции 2015 году. Из изученных локусов, только rs344435545 обладает потенциалом изменения функции белка. **Выводы.** Имеющая влияние на функцию белка неблагоприятный аллель T в rs344435545, не присутствует в латвийской белой породе свиней. За девять лет в фермерских хозяйствах у латвийской белой породы свиней произошла потеря изменчивости rs323041392. Как латышской так и европейской животноводческой отрасли необходимо учесть, что интенсификация размножения может снизить генетическое разнообразие, конкретные рабочие характеристики и адаптивность к экологическим проблемам у местных пород в небольших популяций.

Ключевые слова: RYR1, полиморфизм, генетическое разнообразие, свиньи.

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