COBISS: 1.08
Agris category code: L10

GENETIC VARIABILITY OF microRNA GENES IN FARM ANIMALS

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ABSTRACT

MicroRNAs (miRNAs) are a class of non-coding RNAs that plays an important role in posttranscriptional regulation of target genes. Regulation requires complementarity between the target mRNA and the miRNA seed region, which is responsible for their recognition and binding. Previous studies in human and mouse have shown that variability of miRNA gene (miR-SNPs) might interfere with its function resulting in phenotypic variation. Polymorphisms within miRNA genes could represent biomarkers for phenotypic traits important in farm animals. The aim of this study was to: 1) update previously developed web-based tool for identification of polymorphisms within miRNA genes (miRNA SNiPer), 2) systematically collect polymorphisms of miRNA genes in pig, cattle, chicken, and horse, and 3) experimentally validate SNPs within miRNA seed regions (miR-seed-SNPs) in cattle. Using miRNA SNiPer tool, polymorphisms within 32 mature miRNA regions, including 12 miR-seed-SNPs, were identified in pig, cattle, and chicken. Bovine miR-seed SNPs were chosen for experimental validation. The bta-mir-2313 locus was shown to be very polymorphic, therefore we validated one SNP with previously unknown validation status within the mature seed region in population of Slovenian Simmental cattle. Additionally, two SNPs in corresponding pri-miRNA were identified. Results of this study can serve researchers for follow up hypothesis-driven experimental studies to evaluate the phenotypic effect of identified miRNA genetic variability in vertebrates.

Keywords: farm animals / biomarkers / microRNA / genetic variability

1 INTRODUCTION

MicroRNAs (miRNAs) are non-coding RNA molecules with approximately 21 nucleotides in length that play an important role in posttranscriptional regulation of mRNA. By binding to the different target gene regions, *i.e.*, 3' untranslated region (3'UTR), 5'UTR, promoter, or coding sequences, they repress or activate translation (reviewed in Kunej *et al.*, 2012). MicroRNA biogenesis begins in the nucleus with the primary transcript (pri-miRNA) of several hundreds or thousands base pairs in length that is cleaved by the action of endonuclease DROSHA to 60 to 70 nucleotides long precursor miRNA (pre-miRNA). Pre-miRNA, with its characteristic stem-loop structure (Fig. 1) is then transported to the cytoplasm where endonuclease DICER cleaves both duplex chains to form mature miRNA (Lee *et al.*, 2002; Bartel, 2004). Products of DICER action also include complementary sequences of mature miRNAs, which are referred to as miRNA* (Lau *et al.*, 2001), and are usually transcribed in lower percentage as mature miRNAs (Lim *et al.*, 2003). The key binding location for translational suppression, also called the seed region, resides in the mature miRNA sequence, more accurately situated at position 2–7 or 2–8 nucleotides from the 5' end of the miRNA (Sun *et al.*, 2009).

Changes in the miRNA expression profile were linked with several diseases (reviewed in Ferdin *et al.*, 2011). Moreover, single nucleotide polymorphisms (SNPs) within 1) miRNA genes, 2) miRNA targets or in

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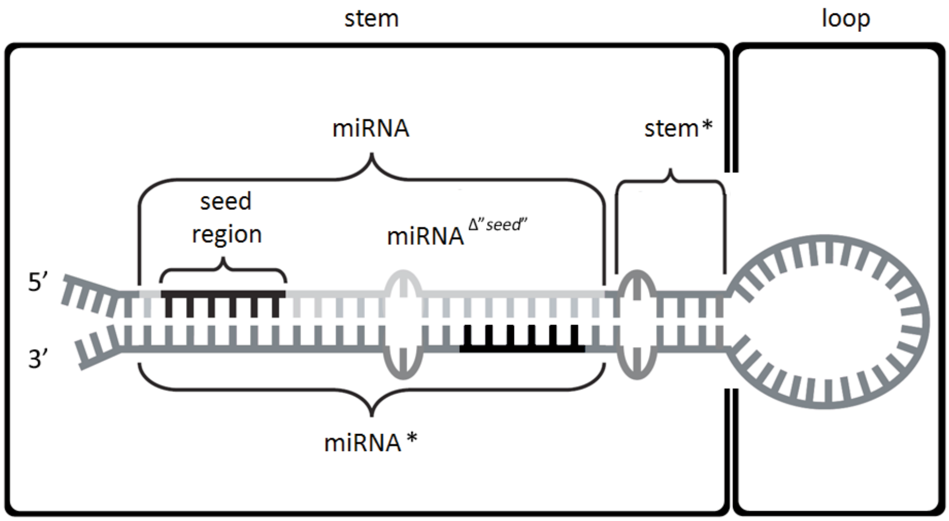

Figure 1: *Secondary structure of miRNA and an example of miRNA stem-loop (adapted from Saunders et al., 2007)*

3) protein-coding genes involved in miRNA biogenesis result in phenotypic differences and therefore affects (associated with) production traits and susceptibility to diseases (Georges *et al.*, 2007). Previously, we developed a web based tool miRNA SNiPer for the identification of polymorphisms residing within miRNA genes (Zorc *et al.*, 2012). Since some farm animals (pig, cattle, chicken, sheep, etc.) represent both – a source of food and disease models, the biomarkers based on miRNA polymorphisms could contribute to the study of phenotypic properties in farm and in medicine. In this study we systematically collected polymorphic miRNA genes from four farm animal species (pig, cattle, chicken, and horse) and further experimentally validated miRNA SNPs in cattle.

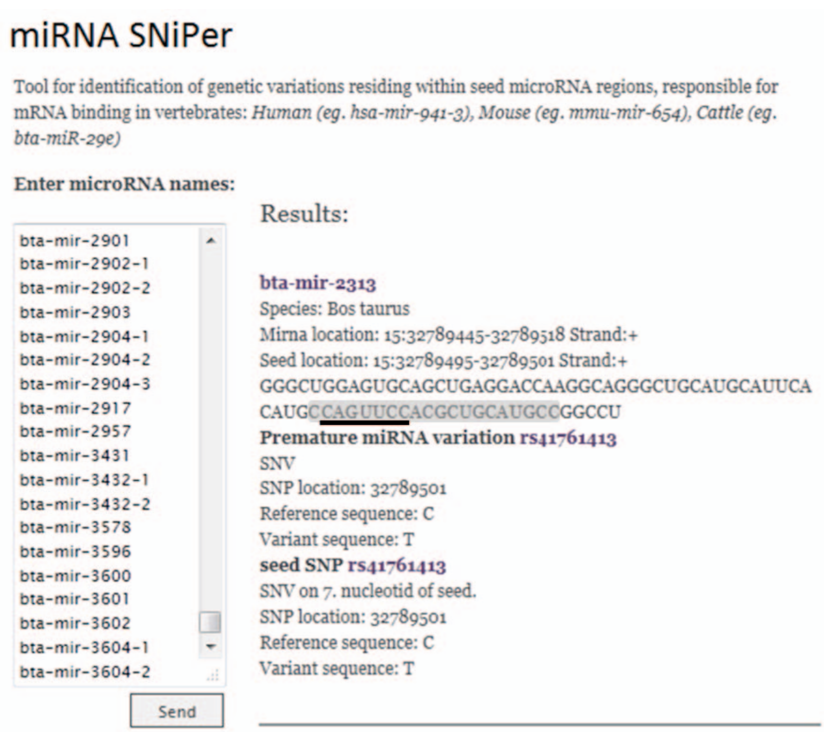

Figure 2: *An example of predicted genetic variability within miRNA in cattle (bta-mir-2313), using a web-based tool miRNA SNiPer. Gray: mature miRNA, underlined region: seed region*

2 MATERIALS AND METHODS

2.1 UPDATE OF THE ONLINE TOOL FOR THE DETECTION OF GENETIC VARIATIONS WITHIN MIRNA GENES.

Previously developed tool miRNA SNiPer was updated with the latest versions of miRBase (release 18; http://www.mirbase.org/) (Kozomara and Griffiths-Jones, 2011), TargetScan (release 5.2; http://www.targetscan.org/) (Lewis *et al.*, 2005), and Ensembl Variation database for pig (Sscrof10.2), horse (EquCab2), cattle (Btau4.0), and chicken (WASHUC2).

2.2 SAMPLES, SEQUENCING OF A DNA PANEL OF SIRES AND VALIDATION OF MIR-SNPS IN CATTLE

Animals were selected from the National progeny test for Slovenian Simmental cattle. DNA samples were extracted from frozen semen of sires using DNeasy Blood & Tissue DNA extraction kit (QiaGen, Düseldorf, Germany). Three bovine miR-seed-SNPs were chosen for experimental validation. Primers were selected using online tool Primer3 (Rozen and Skaletsky, 2000). Due to presence of the *repetitive sequence,* primers in region of SNP rs42658514 within bta-mir-2450c couldn't be designed. For experimental validation, the following primers were used: for bta-mir-2313 forward primer (F) 5'-GCACA-GACTCTCAGCCACTG-3' and reverse primer (R) 5'-CTGACTGAGGCTCTCGCTCT-3', and for bta-miR-29e (F) 5'-TGTAGGGACTGGTTGTGGAA-3' and (R) 5'-TCTACTGAACACAGCCCCATC-3'. PCR products were purified using *Exo*I (Exonuclease I) and SAP (shrimp alkaline phosphatase (both Fermentas, Vilnius, Lithuania) and following sequencing reaction for capillary electrophoresis on ABI3130xl.

3 RESULTS AND DISCUSSION

Previously developed web-based tool miRNA SNiPer, designed for the detection of genetic variations within miRNA genes in vertebrates, was updated. The tool accepts a list of miRNA genes and returns a table of variations within different regions of miRNA genes: pre-miRNA, mature, and seed region (Fig. 2).

The number of total known miRNAs and putative SNPs, as well as the list of miR-SNPs for four farm animal species (pig, horse, cattle, and chicken) is presented in table 1. The highest number of SNPs in farm animals is currently known for chicken and cattle, while the numbers for other farm animals are significantly lower. Thirty-two polymorphisms overlapping mature miRNAs, including 12 within seed region (miR-seed-SNPs), were identified. One miR-seed-SNP was identified in pig, three in cattle, and seven in chicken. Bovine miR-seed-SNPs were selected for experimental validation in eight Slovenian Simmental cattle sires.

All collected miR-seed-SNPs have an unknown

***Table 1:** Number of known miRNAs and SNPs within four farm species (pig, cattle, chicken, and horse) and list of SNPs within miRNA mature and seed region*

Species	Total number of known miRNAs	Total number of SNPs	No. of SNPs within seed/mature miRNA region	miRNA comprising seed SNP	miR-seed-SNP ID
Pig	228	545.950	1/8	ssc-mir-4335	rs80984906
Cattle	662	2.201.071	3/9	bta-mir-29e	rs41825418
				bta-mir-2313	rs41761413
				bta-mir-2450c	rs42658514
Chicken	499	3.292.991	8/14	gga-miR-1568	rs14511527
				gga-miR-1614*	rs15172520
				gga-miR-1644	rs14076349
				gga-miR-1648*	rs14281065
				gga-miR-1657	rs14934924
				gga-miR-1658	rs16681031
					rs16681032
					rs16681033
Horse	341	1.163.258	0/1	/	/

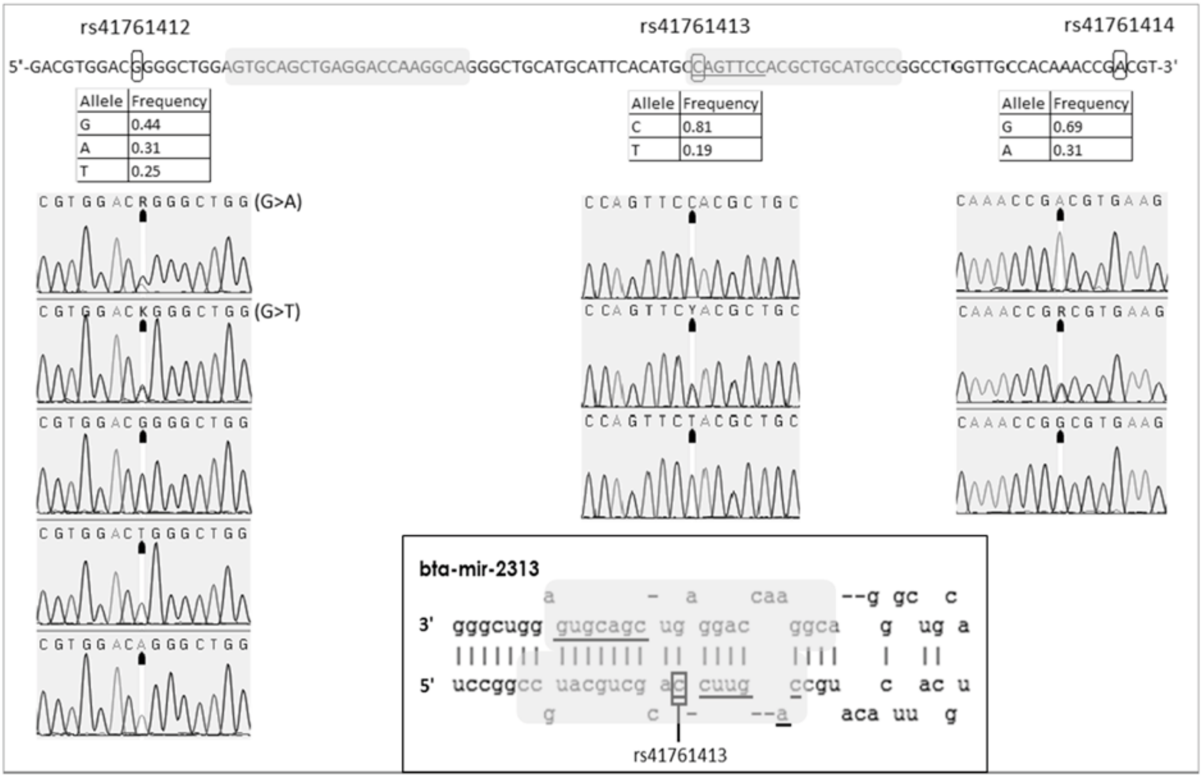

Figure 3: *Validation of three pri-miRNA SNPs located within bta-mir-2313 in cattle. Gray: mature miRNA, underlined region: seed region*

validation status according to the NCBI database and have not been genotyped yet. Experimental validation revealed that polymorphism rs41825418 within bta-mir-29e was monomorphic, while miR-seed-SNP rs41761413 within bta-mir-2313 was polymorphic in analyzed population of Slovenian Simmental cattle (Fig. 3). Additional two SNPs in corresponding pri-miRNA were identified: rs41761412 with poly-allelic substitution (G> T> A) and rs41761414 with substitution (A> G).

4 CONCLUSION

Since miRNA polymorphisms may have a profound effect on a wide range of phenotypes, genetic variability of miRNA genes in farm animals was examined. Bioinformatics tool miRNA SNiPer was updated and used for assembling a list of all known miRNA SNPs in four farm species (pig, horse, cattle, and chicken). Our results show, that most of the miR-SNPs still need to be validated. The project is ongoing, as novel miRNAs and SNPs are yet to be discovered in farm animals, as well as in other animal species. Collated data can be used by interested scientific community to help retrieve valuable information and design efficient experimental plans in the field of miR-SNP research. Association study between validated SNPs located within bta-mir-2313 and carcass traits in paternal half-sib families of the Slovenian Simmental cattle is still under way and current results will be presented. This project may yield new findings useful for development of molecular markers in selection programs allowing more effective, marker assisted selection in farm animals.

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