UDC 575
DOI: 10.2298/GENSR1303799Y
Original scientific paper

BIOCOMPUTATIONAL GENOME-WIDE ANALYSIS OF MICRO RNA GENETIC VARIABILITY IN SOME VERTEBRATES

Abdulmojeed YAKUBU[1], Ibrahim Suleiman MUSA-AZARA[2], Blessing N. Saleh YAKUBU[2], Sylvester I. DAIKWO[3], Samuel Ter VINCENT[4], Ojo Michael MOMOH[4] and Ndubuisi I. DIM[4]

[1]Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Lafia, Nasarawa State, Nigeria.
[2]Department of Animal Science, College of Agriculture, Lafia, Nasarawa State, Nigeria.
[3]Department of Animal Science, Federal University, Wukari, Taraba State, Nigeria.
[4]Department of Animal Breeding and Physiology, University of Agriculture, Benue State, Nigeria

Yakubu A., I. S. Musa-Azra, B.N.S. Yakubu, S.I.Daikwo, S.T.Vincet. O.M.Momoh, and N.I.Dim (2013): *Biocomputational genome-wide analysis of microrna genetic variability in some vertebrates*. Genetika, Vol 45, No. 3, 799-810.

MicroRNAs (miRNAs) are small endogenously expressed single-stranded RNAs that regulate gene expression post transcriptionally and shape diverse cellular pathways. miRNAs regulate a wide range of biological processes through the recognition of complementary sequences between miRNAs and their target genes. The present investigation aimed at determining *in-silico* the genetic variability of miRNA genes in some livestock and non-livestock species. Effects of single nucleotide polymorphisms (SNPs) in genes' 3'UTR on target gain/loss of human miRNAs were also explored. A total of twenty four mature miRNA sequences and genomic coordinates in three livestock [chicken (5), pig (1) and cattle (9)] and two non-livestock (human (6) and mouse (3)] species were retrieved from the miRBase 15 release. Computational scanning of polymorphisms in the miRNAs revealed 33 and 20 polymorphic sites in livestock and non-livestock species, respectively. Of this, 7 (chicken), 11 (cattle) and 2 (mouse) were located within the seed region. The *de novo* computational prediction revealed that SNPs rs1042725 (C/U) and rs1044129 (A/G) in genes' 3'UTR of human miRNAs positively influenced the

Corresponding author: Abdulmojeed Yakubu, Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, P.M.B. 135, Lafia, Nasarawa State, Nigeria, Mobile No*: +2348065644748, Email: abdulmojyak@gmail.com*

target site thereby resulting in target gain. However, the effects of SNPs rs56109847 (A/G), rs28927680, rs12720208 (G/A) and rs5186 (A/C) were negative. The evolutionary tree showed that the relationship between miRNA consensus sequences of livestock (pig, chicken and cattle) was closer compared to non-livestock species (mouse and human), which could be implicated in morphological complexity among vertebrates. Although the function of miRNA is only beginning to be understood, future *in-silico* research evaluating the functional effect of miRNA in gene translation and subsequent biological pathways especially in livestock is of paramount importance; and this should be complemented with hypothesis-driven experimental studies to evaluate the phenotypic effect of identified miRNA genetic polymorphisms in animals.

Key words: miRNAs, polymorphisms, *in-silico*, functional effect, animals.

INTRODUCTION

The first microRNA was discovered in *Caenorhabditis elegans* in 1993, and since then, thousands of microRNAs (miRNAs) have been identified from almost all eukaryotic organisms examined (MENG *et al*., 2013). They are a growing class of small non-coding RNA molecules, 18-25 nucleotides in length, and important post-transcriptional regulators of target mRNAs. Initially, miRNAs are processed from transcript and form hairpin-like loops (WINTER *et al*., 2009). By binding (mature miRNAs) to the different target gene regions, that is, 3′-untranslated region (3′-UTR), 5′-UTR, promoter or coding sequences, they repress or activate translation (KUNEJ *et al*., 2012, ZHANG *et al*., 2013). Mutation, dysfunction, and/or dysregulation of miRNAs may give rise to diseases such as coronary artery disease, cancer, diabetes, AIDS, hepatitis, and obesity in humans. In animals, miRNAs are important genetic regulators. A small number of miRNAs are associated with known physiological roles such as muscle and organ development, the immune response, stress response, metabolism and longevity (MCDANELD, 2009; LIU *et al*., 2010). Animal miRNAs appear to have expanded in conjunction with an escalation in complexity during early bilaterian evolution. Their small size and high-degree of similarity makes them challenging for phylogenetic approaches (GUERRA-ASSUNÇÃO and ENRIGHT, 2012).

Previous studies have shown that miRNA gene variability can interfere with its function, resulting in phenotypic variation. Polymorphisms within miRNA genes present a source of novel biomarkers for phenotypic traits in animal breeding (SKOK *et al*., 2013). Each miRNA is potentially capable of regulating hundreds (or even thousands) of mRNA targets simultaneously. It is therefore important that their regulation be tightly controlled. Moreover, it has been postulated that intronic miRNAs may regulate the same biological pathway as their host genes. Several examples of this have been found, namely in the regulation of Myosin expression (VAN ROOIJ *et al*., 2009) and cholesterol biosynthesis (RAYNER *et al*., 2011). MicroRNAs' effects on target gene expression can be roughly classified into two types: “tuning” and “buffering”. Tuning relates to effects on the target gene expression level, whereas buffering relates to repression of expressional variation (WU *et al*., 2009). It is speculated that the dual functions of miRNAs could represent two stages in miRNA evolution, miRNA initially acting by reducing

variance in gene expression, and only gradually taking on tuning of the expressional level over time (WU *et al*., 2009). Apparently, miRNAs of varying age are not “equal”, as older miRNAs are commonly more highly and broadly expressed than younger miRNAs (LU *et al*., 2008), and knockout of an older miRNA results in a more severe phenotype than knockout of a younger miRNA (VAN ROOIJ *et al*., 2007).

The present study therefore, aimed at determining *in-silico* the genetic variability of miRNA genes in some livestock and non-livestock species. It also examined the effects of single nucleotide polymorphisms (SNPs) in genes' 3'UTR on target gain/loss of human miRNAs.

MATERIALS AND METHODS

A total of twenty four mature miRNA sequences and genomic coordinates in three livestock [chicken (5), pig (1) and cattle (9)] and two non-livestock (human (6) and mouse (3)] species were retrieved from the miRBase 15 release (http://www.mirbase.org/) (GRIFFITHS-JONES *et al*., 2006). Annotation of genomic position for miRNAs in these five species was also extracted from miRbase. The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR) (KOZOMARA and GRIFFITHS-JONES, 2011).

The upgraded bioinformatics tool MIRNA SNIPER 3.0 (SKOK *et al*., 2013) was used for assembling a list of some known miRNA SNPs in all the five species investigated. MIRNA SNIPER was developed for the detection of polymorphisms residing within miRNA genes in vertebrates. It accepts a list of miRNA genes and returns a table of variations within different regions of miRNA genes: pre-miRNA, mature, and seed region (ZORC *et al*., 2012). The mature sequences are designated as ‘‘miR’’ and the precursor hairpins as ‘‘mir’’ (GRIFFITHS-JONES *et al*., 2006). The tool retrieves data from multiple sources such as miRNA gene sequences, genomic coordinates, and nomenclature from miRBase, release 18 (http://www.mirbase.org/) (KOZOMARA and GRIFFITHS-JONES, 2011); locations of miRNA seed regions from TargetScan, release 5.2 (http://www.targetscan.org/) (LEWIS *et al*., 2005), and locations of genetic polymorphisms from Ensembl Variation database, release 64 (http://www.ensembl.org/) (MCLAREN *et al*., 2010).

The likelihood of a particular SNP to affect miRNA target sites was estimated using web-based application, miRNASNP 2.0, which predicts the functional impact of a SNP on putative microRNA targets. This application interrogates the 3'-untranslated region and predicts if a SNP within the target site will disrupt/eliminate or enhance/create a microRNA binding site (GONG *et al*., 2009). This was specifically done for human miRNA sequences.

Multiple sequence alignments were constructed on the basis of the pairwise alignments. Phylogenetic analyses of the mature miRNA sequences of the species were analyzed using the MEGA package (TAMURA *et al*., 2011). The evolutionary history was inferred using the Maximum Parsimony (MP) method. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (NEI and KUMAR, 2000). Similarly, consensus miRNA sequences were used to establish evolutionary relationships among the taxa as adopted in YAKUBU *et al*. (2013). The evolutionary history was inferred using the Minimum Evolution method (RZHETSKY and NEI,

1992). The evolutionary distances were computed using the p-distance method. The Neighbor-joining algorithm was used to generate the initial tree.

RESULTS AND DISCUSSION

SNPs in Pre-mature, mature and seed regions of cattle, chicken, pig, human and mouse are shown in Tables 1 and 2. 33 and 20 polymorphic sites in livestock and non-livestock species, respectively were observed; out of which, 7 (chicken), 11 (cattle) and 2 (mouse) were located within the seed region. A SNP occurring in a pre-miRNA or in the seed region of a miRNA can alter the secondary structure and therefore affect processing of a miRNA to maturity, long ranging phenotypic effects (LIU *et al*., 2009). Large-scale transcriptomic and proteomic studies have revealed that the primary determinant for miR binding is perfect consecutive Watson-Crick base-pairing between the target RNA and the miR at positions 2–7 or 2–8 of the 5' end of the mature miR, often denoted as the “seed” region (The key binding location for translational suppression) (BRENNECKE, 2005; SUN *et al.*, 2009). This signature has been reaffirmed with crystallographic studies of ribonucleoprotein Ago-miR complexes showing that the seed region is organized in a helical conformation that exposes it to base-pair with the target RNA. As expected, miR SNPs in the seed region would ultimately result in the regulation of a completely different set of mRNA targets (DOXAKIS, 2013).

Effects of SNPs in genes 3'UTR on target gain/loss of human miRNAs are shown in Table 3. SNPs rs1042725 (C/U) and rs1044129 (A/G) in genes’ 3'UTR of human miRNAs, positively influenced the target site, thereby resulting in target gain. However, the effects of SNPs rs56109847 (A/G), rs28927680, rs12720208 (G/A) and rs5186 (A/C) were negative. The activity of miRNAs can be affected by single nucleotide polymorphisms (SNPs) that occur either in the miRNA or in the miRNA target site on the mRNA (MISHRA *et al*., 2007). These miRSNPs can alter miRNA gene processing and/or the normal mRNA-miRNA interactions. Thus, these SNPs can create new miRNA target sites or destroy old target sites. Hence, these miRSNPs may also contribute to the interindividual variability in the enzyme expression and activity (RAMAMOORTHY *et al*., 2012). Two recent reports indicate that allelic variation in miRNA target sites have contributed to phenotypic differences in livestock. There is substantial interest in methods designed to predict the miRNA targets and effect of single nucleotide polymorphisms (SNPs) on microRNA binding, given the impact of miRNA on posttranscriptional regulation and its potential relation to complex diseases (ZHU *et al*., 2012). A G to A transition in the 3'UTR of myostatin (GDF8) creates an illegitimate target site for the myogenic miRNAs miR-1 and miR-206 in Texel sheep (CLOP *et al*., 2006; LIU *et al*., 2009). The recognition of the myostatin A allele by miR-1 and miR-206 has been shown to reduce the expression of myostatin, which contributes to the muscle hypertrophy found in these animals. The polled (absence of horns) trait has been mapped to the proximal end of chromosome 1 (GEORGES *et al*., 1993). Computational scanning of polymorphisms in this chromosomal region in Holsteins identified a polymorphism in the 3'UTR of synaptojanin (SYNJ1), which could disrupt the target sites for miRNAs let-7 and miR-98, and it was suggested that this polymorphic miRNA target site may contribute to the polled trait in cattle (CARGILL *et al*., 2008). Therefore, the present SNPs in the 3'UTR of human microRNA may be exploited in future experimental studies.

Table 1. Genetic variability in cattle, chicken and pig showing SNPs in Pre-mature, mature and seed regions

mature miRNA	Sequence	SNP ID	Region/variation
bta-miR-29e	UAGCAUCAUUUGAAAUCAGUGUUU	rs41825418	Seed, SNP (U>C)
bta-miR-2313-5p	AGUGCAGCUGAGGACCAAGGCA	rs41761413	Pre-mature, SNP (C>U)
	CCAGUUCCACGCUGCAUGCC	rs41761413	Seed, SNP (C>U)
bta-miR-2450c	CACGUCAGUAGAGGCGCGUGUG	rs42658514	Seed, SNP (G>A)
bta-miR-2489	AAAUGACAGGGGACAUGAGUUU	rs110544069	Seed, SNP (C>U)
		rs109834057	Seed, SNP (A>G)
		rs136326300	Pre-mature, SNP (C>U)
bta-miR-1260b	AUCCCACCACUGCCACCA	rs133713823	Mature, SNP (U>G)
		rs134960452	Seed, SNP (U>G)
		rs136690815	Pre-mature, SNP (A>G)
		rs134279894	Pre-mature, SNP (U>G)
bta-miR-1291	UGGCCCUGACUGAAGACCUGCAGU	rs110817643	Seed, SNP (C>U)
bta-miR-133a	UUUGGUCCCCUUCAACCAGCUG	rs137070651	Seed, SNP (U>C)
bta-miR-2318	GUGUAUGAUGAAUUAUCUGA	rs134638324	Seed, SNP (A>C)
bta-miR-2369	GUAGGUUGUGGGUUUUUGUUU	rs135405976	Mature, SNP (C>U)
		rs136780194	Seed, SNP (A>G)
		rs134753593	Seed, SNP (A>C)
		rs135082893	Pre-mature, SNP (G>A)
gga-miR-1568	GACUCAUAGAUCUGAAGGCAG	rs14511526	Mature, SNP (U>C)
		rs14511527	Seed, SNP (A>G)
gga-miR-1614	GGCAUGGCAGACUCACCCUGC	rs15172520	Pre-mature, SNP (G>A)
gga-miR-1614*	CAGGGAGGAACUGCCAGCAGA	rs15172520	Seed, SNP (G>A)
gga-miR-1644	UCUGUUGUGCAGGGCUGUGCU	rs14076349	Seed, SNP (U>C)
gga-miR-1648	CGGCUCGGCUCGGCUCCGCUC	rs14281065	Pre-mature, SNP (U>-)
		rs14281066	Pre-mature, SNP (G>A)
		rs14281065	Seed, SNP (U>C)
		rs14281066	Mature, SNP (G>A)
gga-miR-1658	UAUACCACCCCCAGGAGUUCUGC	rs16681031	Pre-mature, SNP (C>G)
		rs16681032	Pre-mature, SNP (C>U)
		rs16681033	Seed, SNP (->G)
gga-miR-1658*	GCUGUGGGUUGGUGUUGAUGG	rs16681031	Seed, SNP (C>G)
		rs16681032	Seed, SNP (C>U)
		rs16681033	Pre-mature, SNP (->G)
ssc-miR-4335	GUGCCCAGCGCUGCAGGGCA	-	-

bta = cattle, gga = chicken, ssc = pig
- = no information

Table 2. Genetic variability in human and mouse showing SNPs in Pre-mature, mature and seed regions

Mature miRNA	Sequence	SNP ID	Region/variation
hsa-miR-96-5p	UUUGGCACUAGCACAUUUUUGCU	rs73159662	Pre-mature, SNP (G>A)
		rs41274239	Pre-mature, SNP (A>G)
hsa-miR-96-3p	AAUCAUGUGCAGUGCCAAUAUG	rs73159662	Pre-mature, SNP (G>A)
		rs41274239	Pre-mature, SNP (A>G)
hsa-miR-184	UGGACGGAGAACUGAUAAGGGU	rs145763978	Pre-mature, SNP (C>G)
		rs41280052	Pre-mature, SNP (G>U)
hsa-miR-299-5p	UGGUUUACCGUCCCACAUACAU	rs41286566	Mature, SNP (C>U)
hsa-miR-299-3p	UAUGUGGGAUGGUAAACCGCUU	rs41286566	Pre-mature, SNP (C>U)
hsa-miR-383	AGAUCAGAAGGUGAUUGUGGCU	rs182042712	Pre-mature, SNP (U>G)
		rs184836993	Mature, SNP (A>G)
		rs112302475	Mature, SNP (U>G)
hsa-miR-34c-5p	AGGCAGUGUAGUUAGCUGAUUGC	-	-
hsa-miR-34c-3p	AAUCACUAACCACACGGCCAGG	-	-
hsa-miR-487a	AAUCAUACAGGGACAUCCAGUU	rs143972054	Pre-mature, SNP (G>A)
mmu-miR-96-5p	UUUGGCACUAGCACAUUUUUGCU	rs29654812	Pre-mature, SNP (C>G)
mmu-miR-96-3p	CAAUCAUGUGUAGUGCCAAUAU	rs29654812	Pre-mature, SNP (C>G)
mmu-miR-654-5p	UGGUAAGCUGCAGAACAUGUGU	rs38443670	Pre-mature, SNP (G>A)
		rs36943496	Seed, SNP (A>G)
mmu-miR-654-3p	UAUGUCUGCUGACCAUCACCUU	rs38443670	Pre-mature, SNP (G>A)
		rs36943496	Pre-mature, SNP (A>G)
mmu-miR-717	CUCAGACAGAGAUACCUUCUCU	rs30372501	Seed, SNP (U>C)
		rs30373504	Pre-mature, SNP (A>G)

hsa = human, mmu = mouse
= no information

The phylogenetic tree depicting evolutionary relationships among the miRNAs of five mammalian species is shown in Figure 1.One of the features observed for mature miRNAs in the present study was their high degree of similarity across species. This is consistent with the findings of PASQUINELLI *et al*. (2000) that many miRNA families have identical mature sequences across a wide range of species. In a related study, GUERRA-ASSUNÇÃO and ENRIGHT (2012) reported that miRNAs have atypical patterns of synteny with preferences for longer clustered regions, which do not appear to be affected by genome compaction. However, there was little specie-specific close proximity [as observed for cattle (bta-miR-2318, bta-miR-29e, bta-miR-133a, bta-miR-1260b, bta-miR-2318, 2313-5p and bta-miR-1291) and chickens (gga-miR-1648-5p and gga-miR-1614-3p in this study] which appeared to be less highly conserved and hence more amenable to phylogenetic approaches. According to GUERRA-ASSUNÇÃO and ENRIGHT (2012), while many miRNAs are present in multiple species and are highly conserved, there are a growing number of miRNAs restricted to specific lineages. However, the

evolutionary pattern changed when consensus miRNA sequence of each species was used to derive the phylogenetic tree (Fig. 2). The relationship between miRNA sequences of livestock (pig, chicken and cattle) was closer compared to non-livestock species (mouse and human).

Table 3. Effect of SNPs in genes' 3'UTR on target gain/loss of human miRNAs

SNP in gene 3'UTR	miRNA	SNP location	Energy change (Kcal/mol)	Effect by SNP on 3'UTR
HTR3E (NM_001256614); rs56109847 (A/G)	hsa-miR-510	chr3:183824557	Wild: -28.00 SNP: 0.00	Loss
HTR3E (NM_198313); rs56109847 (A/G)	hsa-miR-3664-3p	chr3: 183824557	Wild: 0.00 SNP: -16.60	Loss
BUD13 (NM_032725); Rs28927680 (C/G)	hsa-miR-548a-3p	chr11:116619073	Wild: -24.60 SNP: 0.00	Loss
BUD13 (NM_032725); Rs28927680 (C/G)	hsa-miR-1323	chr11:116619073	Wild: -20.80 SNP: 0.00	Loss
BUD13 (NM_032725); Rs28927680 (C/G)	hsa-miR-548o-3p	chr11:116619073	Wild: -18.90 SNP: 0.00	Loss
BUD13 (NM_032725); Rs28927680 (C/G)	hsa-miR-4720-5p	chr11:116619073	Wild: -18.10 SNP: 0.00	Loss
BUD13 (NM_032725); Rs28927680 (C/G)	hsa-miR-4799-3p	chr11:116619073	Wild: -20.80 SNP: 0.00	Loss
HMGA2 (NM_003483); rs1042725 (C/U)	hsa-miR-4742-3p	chr12:66358347	Wild: 0.00 SNP: -9.30	Gain
HMGA2 (NM_003483); rs1042725 (C/U)	hsa-miR-4760-3p	chr12:66358347	Wild: 0.00 SNP: -13.40	Gain
FGF20 (NM_019851); rs12720208 (G/A)	hsa-miR-433	chr12:16850399	Wild: -14.50 SNP: -12.30	Loss
AGTR1 (NM_000685); rs5186 (A/C)	hsa-miR-155-5p	chr12:148459988	Wild: -17.20 SNP: 0.00	Loss
RYR3 (NM_001036); Rs1044129 (A/G)	hsa-miR-4742-3p	chr15:34158266	Wild: 0.00 SNP: -16.60	Gain

Figure 1. Phylogenetic tree showing evolutionary relationship in miRNAs of five mammalian species. Branches corresponding to partitions reproduced in less than 50% trees were collapsed. The consistency index is (0.223684), the retention index is (0.394872), and the composite index is 0.088327 (0.088327) for all sites and parsimony-informative sites (in parentheses.

Analysis of miRNAs phylogenetics could be a useful starting point to explore the molecular basis of morphological complexity (HEIMBERG *et al*., 2008). According to LEE *et al*. (2007), the diversity of the microRNA repertoire, the complexity of their expression patterns, and the diversity of the miRNA targets are correlated with the animal's morphological complexity. Mechanistically, this is more than plausible since the miRNA pathway can influence large gene networks in a coordinated manner and miRNAs are known to be involved in the regulation of nearly all cellular processes (TANZER *et al*., 2008). The evolution of microRNAs is characterized not only by the continuing innovation of novel families but also by the diversification of established families spawning additional paralogous family member. Animal miRNAs are often organized in genomic clusters, usually indicating a single polycistronic primary precursor

transcript, which may carry members of several distinct microRNA families (TANZER *et al*., 2008). Like protein-coding gene families (YAKUBU *et al*., 2012; AJAYI *et al*., 2013) the miRNA families evolved through gene duplications and gene loss; and there is an increasing amount of evidence that whole genome duplication events actually occurred twice during the emergence of vertebrates (DEHAL and BOORE, 2005; LI *et al*., 2008), being a major source of morphological complexity among vertebrates.

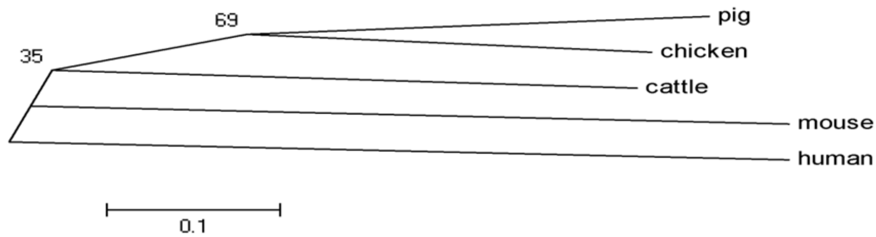

Figure 2. Evolutionary tree derived from consensus miRNA sequences of some vertebrates. The evolutionary history was inferred using the Minimum Evolution method. The optimal tree with the sum of branch length = 1.86250000 is shown.

CONCLUSION

The potential of miRNAs seems not to have been well exploited in vertebrates especially in Nigeria, sub-saharan Africa as there is dearth of information on PCR experimentally validated miRNA studies. Therefore, efforts should be geared towards reasonable use of the validated miRNA SNPS to enhance our understanding of the role of miRNA in regulating key cellular and physiological pathways in both livestock and non-livestock species.

Received August 03th, 2013
Accepted October 05th, 2013

REFERENCES

AJAYI, O.O., M.A. ADEFENWA, B.O. AGAVIEZOR, C.O.N. IKEOBI, M. WHETO, M. OKPEKU, S.A. AMUSAN, A. YAKUBU, M. DE DONATO, S.O. PETERS and I.G. IMUMORIN (2013). A novel *Taq*I polymorphism in the coding region of the ovine *TNXB* gene in the MHC class III region: Morphostructural and physiological influence. Biochemical Genetics (in press), doi10.1007/s10528-013-9622-9.

BRENNECKE, J., A. STARK, R.B. RUSSELL and S.M. COHEN (2005): Principles of microRNA-target recognition. PLoS Biol *3*: e85.

CARGILL, E.J., N.J. NISSING and M.D. GROSZ (2008): Single nucleotide polymorphisms concordant with the horned/polled trait in Holsteins. BMC Research Notes *1*: 128 doi:10.1186/1756-0500-1-128.

CLOP, A., F. MARCQ, H. TAKEDA, D. PIROTTIN, X. TORDOIR, B. BIBE, J. BOUIX, F. CAIMENT, J.M. ELSEN, F. EYCHENNE, C. LARZUL, E. LAVILLE, F. MEISH, D. MILENKOVIC, J. TOBIN, C. CHARLIER and M. GEORGES (2006): A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet *38*: 813-818.

DEHAL, P. and J.L. BOORE (2005): Two rounds of whole genome duplication in the ancestral vertebrate. PLoS Biol *3*: e314.

DOXAKIS, E. (2013): Principles of miRNA-Target Regulation in Metazoan Models. Int J Mol Sci *14*: 16280-16302.

GEORGES, M., R. DRINKWATER, T. KING, A. MISHRA, S.S. MOORE, D. NIELSEN, L.S. SARGEANT, A. SORENSEN, M.R. STEELE, X. ZHAO, J.E. WOMACK and D.J.S. HETZEL (1993): Nat Genet *4*: 206.

GONG, J., Y. TONG, H.M. ZHANG, K. WANG, T. HU, G. SHAN, J. SUN and A.Y. GUO (2012): Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. Human Mutation *33*: 254–263.

GRIFFITHS-JONES, S., R.J. GROCOCK, S. VAN DONGEN, A. BATEMAN and A.J. ENRIGHT (2006): miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res *34*: D140–144.

GUERRA-ASSUNÇÃO, J.A. and A.J. ENRIGH (2012): Large-scale analysis of microRNA evolution. BMC Genomics *13*:218, http://www.biomedcentral.com/1471-2164/13/218

HEIMBERG, A.M., L.F. SEMPERE, V.N. MOY, P.C.J. DONOGHUE and K.J. PETERSON (2008): MicroRNAs and the advent of vertebrate morphological complexity. Proceedings of the National Academy of Sciences *105*(8): 2946–2950.

KOZOMARA, A. and S. GRIFFITHS-JONES (2011): miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Research *39*: D152–D157.

KUNEJ, T., I. GODNIC, S. HORVAT, M. ZORC and G.A. CALIN (2012): Cross talk between microRNA and coding cancer genes. Cancer Journal *18*: 223–231.

LEE, C.T., T. RISOM, and W.M. STRAUSS (2007): Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. DNA Cell Biol *26*: 209–218.

LEWIS, B.P., C.B. BURGE and D.P. BARTEL (2005): Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA Targets. Cell *120*: 15–20.

LI, J., G. MUSSO and Z. ZHANG (2008): Preferential regulation of duplicated genes by microRNAs in mammals. Genome Biology *9*: R132 (doi:10.1186/gb-2008-9-8-r132).

LIU, H.-C., J. A. HICKS, N. TRAKOOLJUL and S.-H. ZHAO (2010): Current knowledge of microRNA characterization in agricultural animals. Animal Genetics *41*: 225-231.

LIU, G., A. FRIGGERI, Y. YANG, Y.J. PARK, Y. TSURUTA and E. ABRAHAM (2009): miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. Proc Natl Acad Sci USA *106* (37): 15819-15824.

LU, J., Y. FU, S. KUMAR, Y. SHEN, K. ZENG, A. XU, R. CARTHEW and C-I. WU (2008): Adaptive Evolution of Newly Emerged Micro-RNA Genes in Drosophila. Molecular Biology and Evolution *25* (5): 929-938.

MCDANELD, T.G. (2009). MicroRNA: Mechanism of gene regulation and application to livestock. J Anim Sci *87* (E. Suppl.):E21–E28. doi:10.2527/jas.2008 -1303.

MCLAREN, W., B. PRITCHARD, D. RIOS, Y. CHEN, P. FLICEK *et al.* (2010): Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics *26*: 2069–2070.

MENG, L., L. CHEN, Z. LI and Z-X. WU (2013): Roles of microRNAs in the *C. elegans* nervous system. Journal of Genetics and Genomics *40*: 445-452.

MISHRA, P.J.,R. HUMENIUK, P.J. MISHRA, G.S. LONGO-SORBELLO, D. BANERJEE and J.R. BERTINO (2007): A miR-24 microRNA binding-site polymorphism indihydrofolate reductase gene leads to methotrexate resistance. Proc Natl Acad Sci USA *104*:13513–13518.

NEI, M. and S. KUMAR (2000). Molecular Evolution and Phylog*enetics*. Oxford University Press, New York.

PASQUINELLI, A.E., B.J. REINHART, F. SLACK, M.Q. MARTINDALE M.I. KURODA, B. MALLER, D.C. HAYWARD, E.E. BALL, B. DEGNAN, P. MÜLLER, J. SPRING, A. SRINIVASAN, M. FISHMAN, J. FINNERTY, J. CORBO, M. LEVINE, P. LEAHY, E. DAVIDSON and G. RUVKUN (2000): Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature *408*:86–89.

RAMAMOORTHY, A., L. LI, A. GAEDIGK, L. D. BRADFORD, E. A. BENSON, D. A. FLOCKHART and T. C. SKAAR (2012): In Silico and In Vitro Identification of MicroRNAs That Regulate Hepatic Nuclear Factor 4α Expression. DMD *40*:726–733.

RAYNER, K.J., C.C. ESAU, F.N. HUSSAIN, A.L. MCDANIEL, S.M. MARSHALL, J.M. VAN GILS, T.D. RAY, F.J. SHEEDY, L. GOEDEKE, X. LIU, O.G. KHATSENKO, V. KAIMAL, C.J. LEES, C. FERNÁNDEZ-HERNANDO, E.A. FISHER, R.E. TEMEL and K.J. MOORE (2011): Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature *478*:404–407.

RZHETSKY, A. and M. NEI (1992): A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution *9*: 945-967.

SKOK, J., D.I. GODNIC, M. ZORC, S. HORVAT, P. DOVC, M. KOVAC and T. KUNEJ (2013): Genome-wide in silico screening for microRNA genetic variability in livestock species. Animal Genetics (in press), doi: 10.1111/age.12072.

SUN, G., J. YAN, K. NOLTNER, J. FENG, H. LI, D.A. SARKIS, S.S. SOMMER and J.J. ROSSI (2009): SNPs in human miRNA genes affect biogenesis and function. RNA *15*: 1640–1651.

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI and S. KUMAR (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731-2739.

TANZER, A., M. RIESTER, J. HERTEL, C.I. BERMUDEZ-SANTANA, J. GORODKIN, I. L. HOFACKER and P. F. STADLER (2008).Evolutionary genomics of micrornas and their relatives. John Wiley & Sons, Inc., pp. 1-35.

VAN ROOIJ, E., L.B. SUTHERLAND, X. QI, J.A. RICHARDSON, J. HILL and E.N. OLSON (2007): Control of Stress-Dependent Cardiac Growth and Gene Expression by a MicroRNA. *Science 316* (5824): 575-579.

VAN ROOIJ, E., D. QUIAT, B.A. JOHNSON, L.B. SUTHERLAND, X. QI, J.A. RICHARDSON, R.J. KELM and E.N. OLSON (2009): A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev Cell *17*: 662–673.

WINTER, J., S. JUNG, S. KELLER, R.I. GREGORY and S. DIEDERICHS (2009): Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol *11*(3): 228–234.

WU, C-I., Y. SHEN and T. TANG (2009): Evolution under canalization and the dual roles of microRNAs—A hypothesis. Genome Research *19* (5)**:** 734-743..

YAKUBU, A., A.E. SALAKO, M. DE DONATO, M.I. TAKEET, S.O. PETERS, M.A. ADEFENWA, M. OKPEKU, M. WHETO, B.O. AGAVIEZOR, T.M. SANNI, O.O. AJAYI, G.O. ONASANYA, O.J. EKUNDAYO, B.M. ILORI, S.A. AMUSAN and I.G. IMUMORIN (2013): Genetic diversity in Exon 2 at the major histocompatibility complex DQB1 locus in Nigerian indigenous goats. Biochemical Genetics *51*: 954-966.

YAKUBU, A., M. DE DONATO, M.I. TAKEET, S.O. PETERS, M.A. ADEFENWA, M. OKPEKU, M. WHETO, B.O. AGAVIEZOR, T.M. SANNI, O.O. AJAYI, S.A. AMUSAN, A.E. SALAKO and I.G. IMUMORIN (2012). Polymorphism Identification in Interleukin-2 Gene in Nigerian Goats. 20th International Conference on Plant and Animal Genomes (Plant and Animal Genomes XX), San Diego, CA. January 14 -18. 2012.

ZHANG, Z., L. HU and X. KONG (2013): MicroRNA or NMD: Why have two RNA silencing systems? Journal of Genetics (in press), http://dx.doi.org/10.1016/j.jgg.2013.09.002.

ZHU, Y., G. SKOGERBØ, Q. NING, Z. WANG, B. LI, S. YANG, H. SUN and Y. LI (2012): Evolutionary relationships between miRNA genes and their activity. BMC Genomics *13*: 718 doi 10.1186/1471-2164-13-718.

ZORC, M., D.J. SKOK, I. GODNIC, G.A. CALIN, S. HORVAT, Z. JIANG, P. DOVC and T. KUNEJ (2012): Catalog of MicroRNA Seed Polymorphisms in Vertebrates PLoS ONE *7* (1): e30737.

BIOKUMPJUTIZOVANA ANALIZA GENOMA KROZ VARIJABILNOSTI MICRO RNK KOD NEKIH KIČMENJAKA

Abdulmojeed YAKUBU[1], Ibrahim Suleiman MUSA-AZARA[2], Blessing N. Saleh YAKUBU[2], Sylvester I. DAIKWO[3], Samuel Ter VINCENT[4], Ojo Michael MOMOH[4] i Ndubuisi I. DIM[4]

[1] Odelenje za nauku o životinjama, Poljoprivredni fakultet, Nasarava Univerzitet, Keffi, Lafia, Nasarawa, Nigeria.

[2] Odelenje za nauku o životinjama, Poljoprivedni koledž, Lafia, Nasarava, Nigerija

[3] Odelenje za nauku o životinjama, federalni Univerzitet, Wukari, Taraba, Nigeria

[4] Department of Animal Breeding and Physiology, University of Agriculture, Benue State, Nigeria

Izvod

Mikro RNKs (miRNKs) regulišu veliki broj bioloških procesa preko prepoznavanja komplementarnih sekvenci između miRNKs i njihovog ciljnog gena. Ispitivanja su obuhvatila *in - silico* genetičke varijabilnosti miRNK kod domaćih i drugih životinjskih vrsta. Vršena su ispitivanja efekta polimorfizma pojedinačnih nukleotida (SNPs) u genima 3 UTR na cilj povećanje/gubitak humanih miRNKs. Kompjutersko skaniranje polimorfizma u mRNKs je potvrdilo 33 polimorfna mesta kod domaćih životinja i 20 kod ostalih animalnih vrsta. Of this, 7 (chicken), 11 (cattle) and 2 (mouse) were located within the seed region. Evoluciono stablo pokazuje da je odnos između miRNKs sekvenci kod domaćih životinja bliži u poređenju sa mišem i čovekom što može da se uključi u morfološku kompleksnost kičmenjaka. Iako je razumevanje funkcije miRNKs na početku buduća *in silico* istraživanja i vrednovanje funkcionalnog efekta miRNKs u translaciji i procesima posle translacije, posebno kod domaćih životinja su od velikog značaja.

Primljeno 03. VIII 2013.
Odobreno 05. X. 2013.