

Letter to Editor

The *Glypican 3*-Hosted Murine *Mir717* Gene: Sequence Conservation, Seed Region Polymorphisms and Putative Targets

Tanja Kunej^{1*}✉, Dasa Jevsinek Skok^{1*}, Simon Horvat^{1,2}, Peter Dovc¹, Zhihua Jiang³✉

1. Department of Animal Science, University of Ljubljana, Groblje 3, SI- 1230 Domzale, Slovenia

2. National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

3. Department of Animal Sciences, Washington State University, Pullman, Washington 99164-6351, USA

*Authors contributed equally to the work.

✉ Corresponding author: Dr. Tanja Kunej, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Domzale, Slovenia. Email: tanja.kunej@bf.uni-lj.si. Dr. Zhihua Jiang, Department of Animal Sciences, Washington State University, Pullman, WA 99164-6351. Email: jiangz@wsu.edu

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Abstract

Mir717 (*mmu-mir-717*) was first reported in mouse and resides in the intron 3 of glypican 3 (*Gpc3*) gene. Our present study revealed that this microRNA (miRNA) gene is conserved among 26 mammalian species and harbors polymorphic sites within the mature seed region in mice. Our finding represents a rare four layer genomic overlap consisting of growth associated quantitative trait locus (QTL), body mass associated *Gpc3* gene, highly conserved miRNA gene and mature miRNA seed single nucleotide polymorphism (SNP) identified in the lean mouse strain 129/Sv. Additionally, genes potentially targeted by *Mir717* include 91 genes associated with obesity and related phenotypes in mammals. Our analysis provides a basis for further experiments to causally connect the identified SNP and *Mir717* gene itself to obesity regulation. Furthermore, our bioinformatics analysis now enables functional annotation of *Mir717* orthologs in other species, thus determining the effect of its target genes on fat-related traits.

Key words: Obesity, *glypican 3* (*Gpc3*) gene, microRNA (miRNA), *Mir717* (*mmu-miR-717*), comparative genomics.

MicroRNA (miRNA) *Mir717* (*mmu-mir-717*) was first reported in mouse, which is located in the intron 3 of the glypican 3 (*Gpc3*) host gene on X chromosome (<http://www.mirbase.org/>). The human miR-717 is also expressed, and plays important regulatory roles in renal osmoregulation [1]. It has been shown that intronic miRNAs could be coordinately expressed with their host gene mRNA, implying that they might share a common transcriptional mechanism, however, anti-correlative miRNA/host gene expression have also been demonstrated [2-4]. Glypican 3 is a member of the glypican-related integral membrane proteog-

lycan family. The protein binds to and inhibits the dipeptidyl peptidase activity of CD26, thus inducing apoptosis in certain cell types. In human, mutations in *GPC3* gene cause the Simpson-Golabi-Behmel overgrowth syndrome [5], whereas *Gpc3* knockout mice show increased body mass, renal dysplasia, and perinatal mortality [6]. Additionally, a QTL for growth was also mapped to the region containing *Gpc3* [7]. Therefore, the objectives of our present study were to 1) elucidate evolutionary conservation of *Mir717* among the mammalian species, 2) identify genetic variants of the *Mir717* in mice, and 3) identify

potential target genes of Mir717, particularly those that are associated with obesity and related conditions [8].

According to the miRBase 13.0 (<http://microrna.sanger.ac.uk/>) and Ensembl (<http://www.ensembl.org/index.html>) databases, the *Mir717* resides within the intron 3 of the *Gpc3* gene (Figure 1A). Orthologs of mouse *Mir717* were retrieved from the Ensembl genome browser using “alignments / multispecies view / eutherian mammals” option. Multiple species sequence alignment was performed using the MultAlin program (<http://bioinfo.genotoul.fr/multalin>). Multiple alignment of known (mouse) and predicted *Mir717* ortholog sequences showed a high level of conserva-

tion among 26 eutherian mammalian species (Figure 1B). So far, the *Mir717* gene has been found to be expressed in the mouse and human only. Due to the high sequence conservation this region could now be analyzed by miRNA prediction algorithms and experimentally to functionally annotate the corresponding orthologous miRNA gene in other species. The mouse mature *Mir717* differs from the corresponding rat and human sequences in only one and two nucleotides, respectively (Figure 1B). Seven species had 100% mature sequence similarity including human (*Homo sapiens*): chimpanzee (*Pan troglodytes*), orangutan (*Pongo pygmaeus*), macaque (*Macaca mulatta*), Philippine tarsier (*Tarsius syrichta*), cat (*Felis catus*) and European rabbit (*Oryctolagus cuniculus*) (Figure 1B).

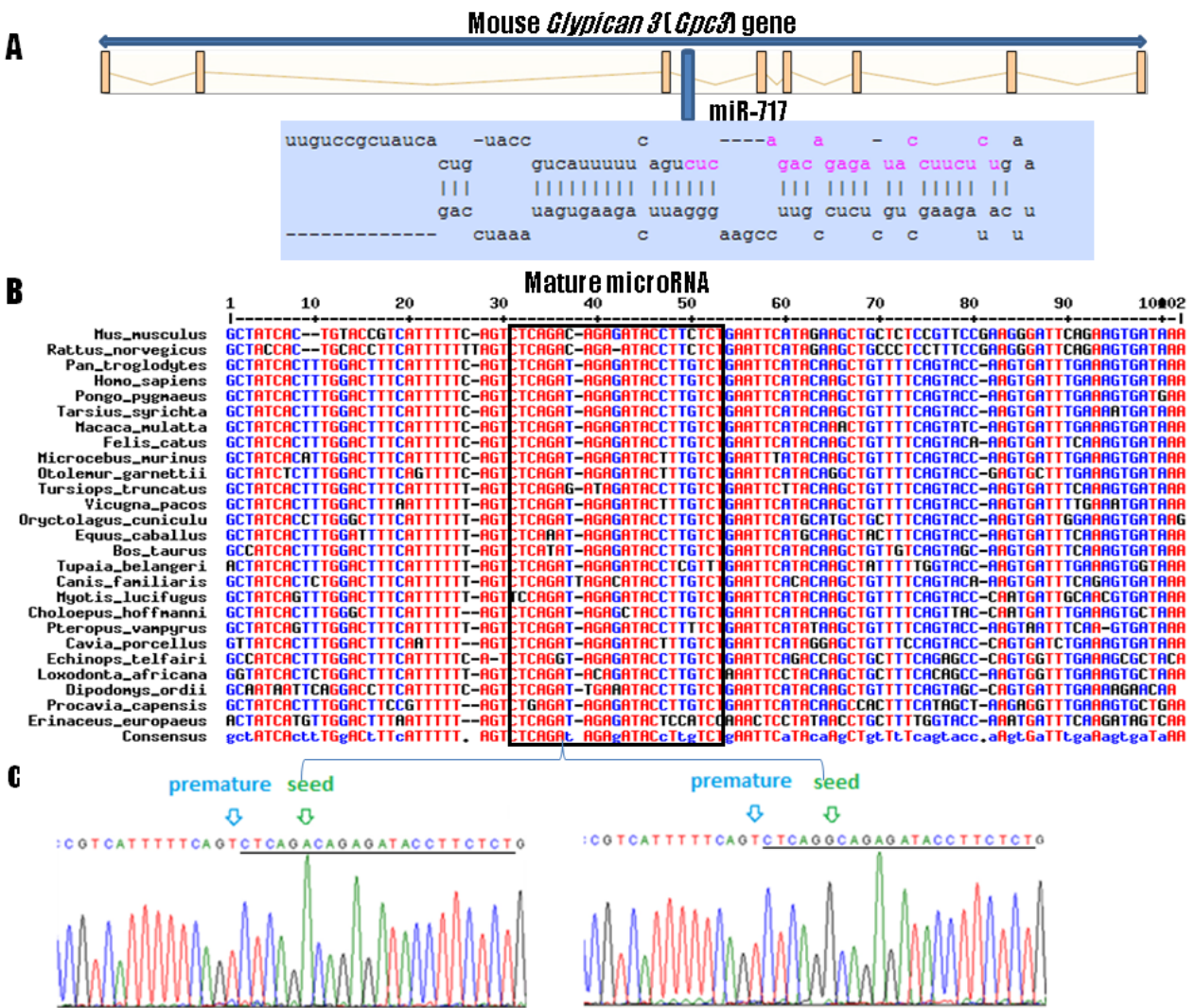


Figure 1. Gene organization, comparative genomics, and genetic variability of the *Mir717* gene. A: genomic organization of the *Gpc3* with *Mir717* in its intron 3. B: *Mir717* alignment among 26 mammalian species, mature miRNA region is marked with the square. C: *Mir717* genetic variability; location of the premature SNP rs30373504 (light blue arrow) and seed SNP rs30372501 (green arrow) polymorphic between the low fat and high fat mouse strains. Mature *Mir717* is underlined.

Genetic variability of *Mir717* in mouse was evaluated by sequencing PCR products (primers *Mir717-F*: CCAAATCACCACCTTTGTCC and *Mir717-R*: AGGAAGCTTGGAGGCAGATT). PCR was carried out in a total volume of 10 μ l including ~50 ng of DNA, 1 \times PCR buffer, 1 mM MgCl₂, 200 μ M dNTPs, 0.5 U *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) and 5 pmol of each primer using Applied Biosystems Gene Amp PCR System 9700 and conditions: 95°C for 10 min, 32 cycles of 94°C for 30 sec, 59°C for 30 sec and 72°C for 30 sec, followed by a further 5-min extension at 72°C. PCR products were treated with *ExoI* and *SAP* enzymes (Fermentas) to remove primers before using a BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems). Capillary electrophoresis was performed on an ABI3130xl genetic analyzer. Sequencing of the 475 bp region flanking the *Mir717* was performed with 2-3 mice per inbred strain: a DNA panel contained the "high growth" mouse mutant (genetic background of C57BL/6J) [9], DBA/2J from Harlan (Italy) and DNA from embryonic stem cell line HM1 isolated from 129/Sv strain [10]. Analysis revealed an A>G substitution SNP within the mature *Mir717* among these three strains at position 49775654 on Chromosome X (Ensembl release 60). The allele A was shared by high fat strains DBA/2J and C57BL/6J. Interestingly, the allele A is also present in 25 out of 26 compared mammalian species (Figure 1B), suggesting an important functional role of this site. However, the 129/Sv strain contained the allele G at the SNP site. As this line exhibits lower values for all obesity related traits (blood lipids, total body fat, weights of individual fat depots etc.; <http://phenome.jax.org/>) this SNP allele G could therefore be potentially associated with leanness in mice. However, to causally confirm this association, segregation analyses should be conducted between the lean line 129/Sv and other high fat strains sharing the SNP allele of C57BL/6J and DBA/2J.

Bioinformatic analysis of *Mir717* was performed using the Ensembl, MGI (<http://www.informatics.jax.org/>) and Patrocles (<http://www.patrocles.org/>) and revealed two SNPs in the *Mir717*. The pre-miRNA SNP (rs30373504) was monomorphic in our tested mouse population, but the polymorphic A>G SNP resided in the mature miRNA (rs30372501). This mature miRNA SNP (miR-SNP) is located within the "seed region"; defined as six or seven nucleotides between the nucleotides 2-7 or 2-8 of the miRNA 5' end that are responsible for mRNA binding [11] (Figure 1C).

The term miR-SNP refers to the variation that occurs in the miRNA gene sequence, whereas miR-TS-SNP refers to the SNP that occurs in the miRNA target site (TS) [11]. MiR-TS-SNPs have been associated with many diseases, including tumor susceptibility [12]. Because one miRNA can have multiple targets, miR-SNPs would be expected to exhibit more profound and broader biological effects than miR-TS-SNPs [11]. The causal effect of the seed miR-SNPs on phenotype variability has been shown recently - two groups discovered that miR-96 seed SNP was responsible for hearing loss in human and mouse [13,14]. Our results further imply the importance of the seed SNP by linking our identified SNP to fat deposition traits.

Additionally, we performed the search for the putative obesity associated *Mir717* targets. Obesity genes were collected from the Human obesity gene map [15] (<http://obesitygene.pbrc.edu>), Rat genome database (RGD) (<http://rgd.mcw.edu/>), Mouse genome informatics (MGI) (<http://www.informatics.jax.org>) and Pubmed (<http://www.ncbi.nlm.nih.gov/>). The search for putative *Mir717* targets was performed using DIANA - microT v3.0 target prediction tool (<http://diana.cslab.ece.ntua.gr/>), TargetScan, Release 5.1 software (<http://www.targetscan.org/>), MirGen (<http://diana.pcbi.upenn.edu/cgi-bin/miRGen/v3/Targets.cgi>), miRDB (<http://mirdb.org/miRDB/>), and PITA (<http://genie.weizmann.ac.il/pubs/mir07/index.html>). We compiled a total of 2610 genes potentially targeted by *Mir717*. The analysis revealed only eight targets discovered by all five tools and they are *2010002N04Rik*, *Camta1*, *Coq4*, *Dedd*, *Dixdc1*, *Spry1*, *Syp*, and *Tyro3*. From the database searches of Human obesity gene map, RGD (rat), MGI (mouse) and Pubmed (cattle) 679 fat deposition related genes were collected. Comparison of *Mir717* target genes to the obesity gene list revealed that 91 target genes were described previously as obesity related.

Several miRNAs have been shown to play an essential role in adipogenesis, such as miR-27 [16], miR-21 [17], miR-27b [18], and miR-335 [19]. The SNP identified in our study is located in the seed region of the miRNA that has to be complementary to the mRNA in order to recognize the target. MiR-SNP could impact on the catalogue of miRNA targets, not only by disrupting the interaction of the mutant *Mir717* with some target genes but also by creating illegitimate targets that are not targeted by the wild type *Mir717*. Our finding represents a four layer genomic overlap: growth associated QTL, body mass

associated *Gpc3* gene, highly conserved miRNA gene and seed miRNA SNP which is a relatively rare event. The analyzed *Mir717* SNP should now be used in future crosses between the low fat strain 129/Sv and the high fat strains DBA/2J and C57BL/6J to test for direct associations of this marker with obesity/leanness related traits. Furthermore, our bioinformatics analysis also provides a basis for functional annotation of *Mir717* orthologs in other species as well as identification of posttranscriptional regulation of fatness-related targets.

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Conflict of Interests

The authors have declared that no conflict of interest exists.

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