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Research Article

Ketogenic Diet Modifies the Expression of MicroRNAs Linked to Migraine

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Abstract

Epidemiological studies have emphasized the relationship between migraine and obesity also pointing out its prevalence in the female sex. The mechanisms promoting pain migraine in obese subjects prone to this neurological disorder is multifactorial, among these the overproduction of soluble mediators favoring neural inflammation, psychological and behavioral risk factors. The ketogenic diet (KD) is a well-recognized as a therapeutic option for refractory pediatric epilepsy and a promising prophylactic treatment for episodic and chronic migraine in the adult. Performing a pilot study on the capability of KD to modulate a plethora of 800 microRNAs (miRs), a group of female obese subjects, 6 of 18 self-reported a reduction of the frequency and the intensity of migraine-pain attacks. Therefore, we check for miRs linked to migraines. The effects of KD seem to be mediated by specific serum hsa-miR-590-5p, hsa-miR-660-3p modulation.

Keywords: *Ketogenic Diet; Migraine; MicroRNAs*

Introduction

Migraine is the most common neurologic disease affecting about 12% of the adult population worldwide with higher prevalence in female sex [1]. According to the International Headache Society (IHS), migraine is classified as a chronic disorder with episodic pain manifestation that became progressive with daily return in some subjects. Hence, identifying risk factors for recurrence has emerged as an important health priority. Several studies have highlighted the interplay between overweight and frequency and severity of migraine attacks and evidences for this relationship have been demonstrated both in adult and pediatric subjects [2-9]. In this regard appropriate lifestyle interventions have been proposed [10]. Ketogenic diet (KD) is a nutritional regimen characterized by less than 30 grams of carbohydrates per day, fostering metabolic changes and optimizing energy metabolism [11] and in obese individuals it allows greater weight loss when compared to other diets [12]. The synthesis of ketone bodies, in this diet regimen positively affects several pathways usually supposed to be part of migraine pathophysiology and in drug refractory epilepsy [13]. In particular, the therapeutic success of KD relies it's the capability to affect brain excitability and metabolism restoring the balance between neuronal activation and inhibition [14]. KD is able to affect microRNAs (miRs) well known epigenetic mediators [22]. To this respect, also the augmented neuronal activity in migraine is able modifies the brain epigenome [16]. Recently the role miRs on the epigenetic modifications occurring in neu-

ropathic pain [17,18] and in migraine [19,20] has been pointed out. These biomolecules belonging to the non-coding RNA-family that suppress target mRNA translation and stability for a wide portion of transcriptome [21]. Delaying protein translation, miRs indirectly modulate several biochemical processes. Our recent study demonstrated that in obese subjects KD is able to deeply affects serum miRs profile linked antioxidant biochemical capacity [23]. In addition, at the final stage of the study six female enrolled obese patients self-reported a better outcome of migraine attack. Based on this observation we analyzed in these subjects miRs profile reported by Andersen., *et al*. to be associated with migraine [31].

Materials and Methods

Population and KD diet

Ethical approval for all human studies was granted in accordance with the Regional Ethics Committee (REC) (#120-18052018). Subjects were also excluded if they showed the presence of hypertension and/or were on medication. The study was considered not to have set up clinical trials and was not registered as such. Written informed consent was obtained from participants which conformed to the standards of ethical practice as outlined in the declaration of Helsinki. The exclusion criteria included diabetes, renal diseases, liver dysfunction, a history of alcohol or drug abuse, and neoplastic diseases in the five years prior to the study. The KD was planned as previously described [19,24]. Briefly, the total caloric intake was set with less than 30g of carbohydrate per day for 3 weeks. Then, the total caloric intake was decreased by further 200 kcal but the carbohydrate amount was rises at 120g per day.

Study endpoint

The primary efficacy endpoint was a statistically significant change (***P < 0.0001) from baseline values of migraine-related miRs expression after 6 weeks of KD program.

RNAs extraction

Total RNA was extracted from 200 µl of blood serum by using miRNeasy Serum/Plasma Kit (QIAGEN). Briefly, samples were lysed with 5 volumes of QIAzol Lysis Reagent for 5 minutes at room temperature. Five µl of exogenous ath-miR-159a and osa-miR-414 (1 nM) spike-in controls (AnaSpec Inc, Fremont-CA-USA) and 200 µl of chloroform were added. The mixture was vortexed, kept at room temperature for 5 minutes, centrifuged at 12000× *g* for 15 minutes at 4°C and the upper (aqueous) phase was collected. Subsequently, 1.5 volumes of 100% ethanol were added to the aqueous phase. The obtained solution was passed through an RNeasy MinElute spin column in sequential 700 µl aliquots, where the total RNA binds to the membrane and phenol and other contaminants were efficiently washed away using specific buffers. Finally, RNA was washed once with 80% ethanol, dried for 2 minutes by centrifugation, and dissolved in 15 µl of RNase-free water. Qubit 4.0 Fluorometer was used for microRNAs quantification using Qubit™ microRNA Assay Kit (Thermo Fisher).

Circulating miRNA profiling through NanoString technology

Expression profile of 100 ng of RNA was performed through NanoString technology by using nCounter Human v3 miRNA Expression Assay Kits (NanoString Technologies) in an nCounter FLEX (Prep Station and Digital Analyzer) (NanoString Technologies), according to manufacturer instructions [25]. Counts of the reporter probes were tabulated for each sample by the nCounter Digital Analyzer. The raw data out- put was imported into nSolver™ [26,27] calculating the geometric mean of the top 100 miRNAs in all samples, effectively normalizing relative to total miRs present [26]. In order to have robust result miRs related to hemolysis and count threshold using average count number of the *spikes in* controls was applied, as previously described [28].

Pathway analysis of miRNA targets by bioinformatics tools

In silico bioinformatics approach using, miR Target Link Human (https://ccb-web.cs.uni-saarland.de/mirtargetlink/), and DIANA miRPath v3.0 (http://snf-515788.vm.okeanos.grnet.gr/). Those databases were employed to detect target genes and miRNA-regulated biological function. String server version 11.0 (https://string-db.org/) was used for target co-expression in human.

Statistical analysis

MiRs profile was analyzed by nSolver™. Statistical analysis was performed by unpaired t-test with Welch's correction or one-way analysis of variance using, GraphPad Prism (San Diego, CA, USA). No power calculation is possible for this pilot study.

Limitation of the study

This is a pilot study and other clinical trials in a large population must be performed to confirm these data.

Results

Subjects characteristic

A total of 6 caucasian female subjects obese in stage 1 of the Edmonton Obesity Staging System (EOSS), underwent to biphasic KD. Subject characteristics were described in table 1.

Characteristic	Obese (n = 6)
Age, y	42.5 ± 5.0
Height, cm	160.1 ± 3.2
Weight, kg	87.5 ± 3.0
BMI, kg/m^2	33.0 ± 0.2

Table 1: Subjects characteristic.

Array of miRs linked to pain-migraine in KD

The miRs profile linked to pain-migraine was analyzed in six female obese subjects reducing their weight during the biphasic KD program and self-reporting the disappearance of migraine attack during but not outside the biphasic diet program. Heatmap and hierarchical clustering based on the most differentially expressed hsa-miRs are shown in figure 1. The signature hsa-miR-590-5p, hsa-miR-211-5p, hsa-miR-26b-5p, hsa-miR-342-5p, hsa-miR-34a-5p, hsa-miR-375, hsa-miR-382-5p, hsa-miR-660-3p. In particular, KD compared to baseline revealed that the serum levels of hsa-miR-211-5p, hsa-miR-26b-5p, hsa-miR-34a-5p and hsa-miR-375 were unchanged before and after the KD program. While a presence in the 66% of subjects was reached for both, hsa-miR-590-5p (4/6), and hsa-miR-660-3p (4/6).

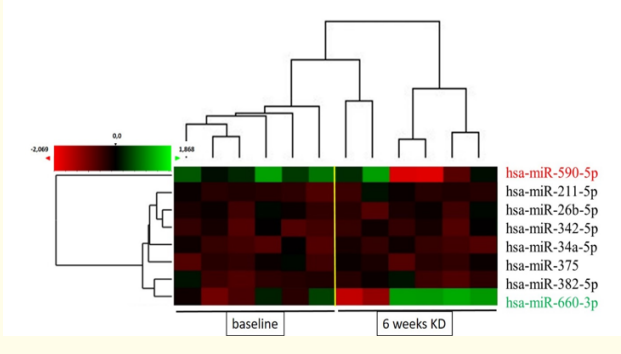

Figure 1: Heat map in log2 data of circulating serum microRNAs linked to pain-migraine at the baseline and after 6 weeks of KD program in 6 female subjects.

Pain linked hsa-miR-590-5p in the serum was found down-regulated (with a P value of P < 0.0001) by KD and the emerging hsa-miR-660-3p was strongly upregulated (with a P value of P = 0.0002) by KD (Figure 2A and 2B) when compared the count number value to the baseline levels.

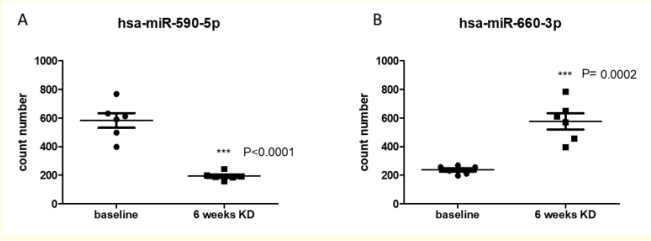

Figure 2: Pain-migraine linked microRNAs in A) hsa -miR-590-5p and B) hsa-660-3p at baseline and after 6 weeks of KD program.

In silico results

Two different databases were used for the in-silico analysis. Data were compared with respect to the number of target genes experimentally validated in both databases. The results are reported in table 2. The number of validated targets found for hsa-miR-590-5p and hsa-miR-660-3p were consistently different. In DIANA miRPath v3.0 program the number of validated target gene was higher in respect to miRtagertLink Human. A total of 68 genes were found for hsa-miR-590-5p of which 2 resulted validated was found in miRtagertLink Human. While, a total of 463 genes was found for hsa-miR-590-5p of which 43 resulted validated in DIANA miRPath v3.0 program. For hsa-miR-660-3p a total of 831 were found in miRtagertLink Human and any validated, while a total of 942 genes for this miR of which 27 validated was unveil by DIANA miRPath v3.0 program (Table 2). Therefore, DIANA miRPath v3.0 program was used for further bioinformatics analysis.

	miRTargetLink Human Database		DIANA tools Database	
	Number of Target Genes	Experimental Validated	Number of Target Genes	Experimental Validated
hsa-miR-590-5p	68	2	463	43
hsa-miR-660-3p	831	0	942	27

Table 2: *In silico* analysis of validated target genes for hsa-miR-590-5p and has-miR-660-3p.

It is worth to note that DIANA miRPath v3.0 program, beside to unveil validated target gene is able to predict miRNA targets with high accuracy based on the DIANA-microT-CDS algorithm that considers the evolutional conservation of miRNA-binding sites in the 3'-UTR region. In table 3 target gene for both miRs are shown. Amongst the validated target gene present in the DIANA miRPath v3.0 program only few were linked to brain or central neurons physiology. Finally, in figure 4 we reported the amount of weight loss of the six female subjects.

miRs gene-target interaction	
hsa-miR-590-5p	hsa-miR-660-3p
YOD1: YOD1 Deubiquitinase	KAT6A: Lysine Acetyltransferase 6A
PBRM1: Polybromo 1	FAM98A: Family with Sequence Similarity 98 Member A
GATAD2B: GATA Zinc Finger Domain Containing 2B	NFIC: Nuclear Factor I C
SKI: SKI Proto-Oncogene	FZR1: Fizzy and Cell Division Cycle 20 Related 1
PLAG1: PLAG1 Zinc Finger	CNTFR: Ciliary Neurotrophic Factor Receptor
CREB5: CAMP Responsive Element Binding Protein 5	APH1A: Aph-1 Homolog A, Gamma-Secretase Subunit
CUX1: Cut Like Homeobox 1	ASH1L: ASH1 Like Histone Lysine Methyltransferase
PTPN14: Protein Tyrosine Phosphatase Non-Receptor Type 14	GIGYF1: GRB10 Interacting GYF Protein 1
PELI1: Pellino E3 UbiquitinProteinLigase 1	NOP9: NOP9 Nucleolar Protein
PPP1R3B: protein phosphatase 1: regulatory subunit 3B.	MYO15A: Myosin XVA
STAG2: Stromal Antigen 2	GRIN2B: Glutamate Ionotropic Receptor NMDA Type Subunit 2B
FNIP2: Folliculin Interacting Protein 2	TSPAN14: Tetraspanin 14
LEMD3: LEM Domain Containing 3	CEP350: Centrosomal Protein 350
ZSWIM6: zinc finger swim – type containing 6	KMT2D: Lysine Methyltransferase 2D
MALT1: Paracaspase	DYNLL2: Dynein Light Chain LC8-Type 2
LATS1: large tumor suppressor kinase 1	GSG2: histone H3 associated protein kinase
GLCCI1: Glucocorticoid Induced 1	HOMER1: Homer Scaffold Protein 1
SAMD9: Sterile Alpha Motif Domain Containing 9	ZNF827: Zinc Finger Protein 827
CPEB3: Cytoplasmic Polyadenylation Element Binding Protein 3	MAFG: MAF BZIP Transcription Factor G
KLF3: kruppel like factor 3	ZBED1: Zinc Finger BED-Type Containing 1
GPR180: G protein-coupled receptor 180	DYNC1H1: Dynein Cytoplasmic 1 Heavy Chain 1
CRIM1: Cysteine Rich Transmembrane BMP Regulator 1	IFNAR2: Interferon Alpha and Beta Receptor Subunit 2
BNIP2: BCL2 Interacting Protein 2	NRBP1: Nuclear Receptor Binding Protein 1
KRIT1: KRIT1 Ankyrin Repeat Containing	CDC23: Cell Division Cycle 23
KLHL15: Kelch Like Family Member 15	KDM5C: Lysine Demethylase 5C
C10orf12: chromosome 10 open reading frame 12	SLC38A1: Solute Carrier Family 38 Member 1
SFXN1: Sideroflexin 1	KLHL29: Kelch Like Family Member 29
TNFRSF11B: TNF Receptor Superfamily Member 11b	
PITHD1: PITH Domain Containing 1	
MRPL30: Mitochondrial Ribosomal Protein L30	

WWP1: WW Domain Containing E3 Ubiquitin Protein Ligase 1	
PCBP1: Poly(RC) Binding Protein 1	
CSTB: Cystatin B	
RNF6: Ring Finger Protein 6	
NRIP1: Nuclear Receptor Interacting Protein 1	
CEP97: Centrosomal Protein 97	
AGO4: Argonaute RISC Component 4	
RAB11A: RAB11A, Member RAS Oncogene Family	
RC3H2: Ring Finger and CCCH-Type Domains 2	
TNFRSF10B: TNF Receptor Superfamily Member 10b	
E2F3: E2F Transcription Factor 3	
PAG1: Phosphoprotein Membrane Anchor with Glycosphingolipid Microdomains 1	
DGKE: Diacylglycerol Kinase Epsilon	

Table 3: Abbreviation and gene name.

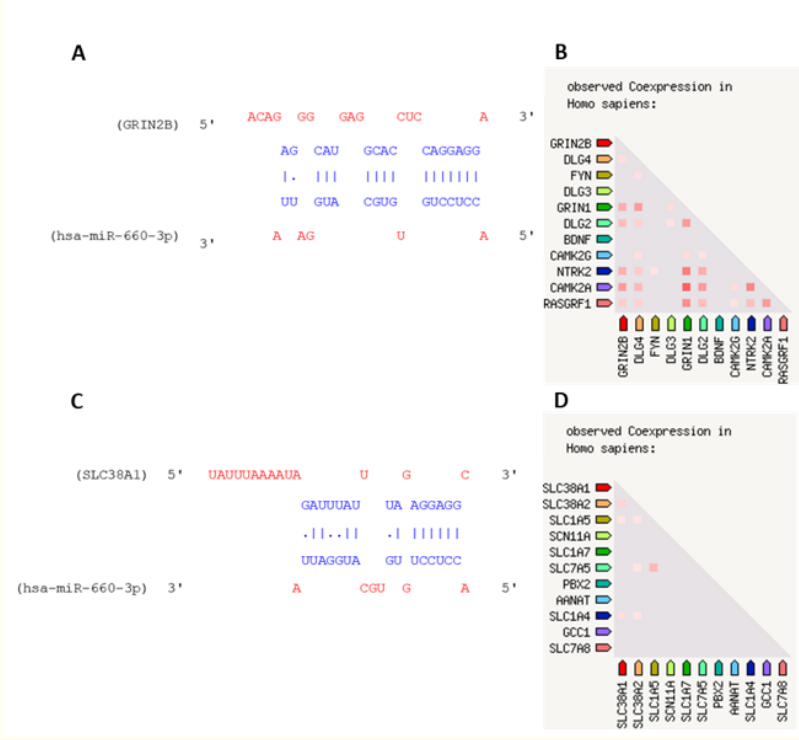

Figure 3: A) hsa-660-3p and GRINB2 interaction; B) GRINB2 co-expression; C) hsa-660-3p and SLC38A1intercation; D) SLC38A1 co-expression.

In figure 3A interaction of experimental validated target gene, GRINB2 3'-UTR-mRNAs and hsa-miR-660-3p is shown as well as its co-expression in human (Figure 3B). The validated target genes found for hsa-miR-660-3p, SLC38A1 and their interaction are shown in figure 3C as well as its co-expression with other human proteins in figure 3D.

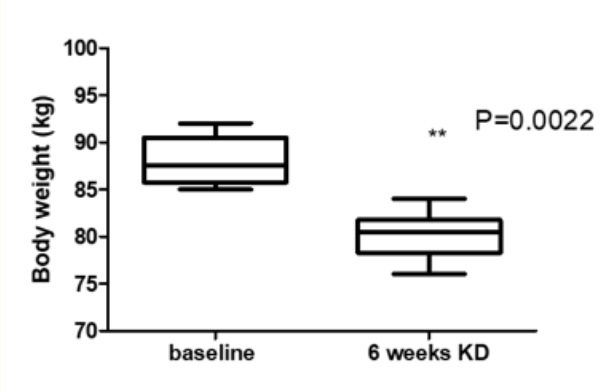

Figure 4: Weight loss from baseline and after 6 weeks of KD program.

Discussion and Conclusion

In the past few years several epidemiological studies [29] highlighted the relationship between migraine and obesity in subjects prone to this neurological disorder also pointing out its prevalence in female sex [30]. The mechanisms underlying this linkage are multifactorial involving the overproduction and release of soluble mediators promoting neural inflammation, physiological and behavioral risk factors. Many of these conditions are affected by weight loss and in this frame the greater efficacy of KD compared to other diet regimens has been demonstrated [12]. We showed that biphasic KD was able to dramatically reduced the frequency and severity of migraine attack in a cohort of female obese subjects (characteristics in table 1). Following to biphasic KD program, we did not record any change in the expression of hsa-miR-211-5p, hsa-miR-26b-5p hsa-miR-34a-5p and hsa-miR-375 (Figure 1). A similar result was also observed for the brain-enriched hsa-miR-382-5p in accordance with previous finding by Andersen., *et al*. [31]. Notably, the miRs network analyzed target a set of different genes encoding proteins involved in ion channels regulation, glutamate neurotransmission or in triggering neurotransmitters release at synapses [31]. This finding suggests that the normalization of cerebral excitability frequently seen in subjects with migraine [14] underwent to KD is likely due to a broad-based action of ketone bodies, β-hydroxybutyrate in particular [13] rather than epigenetic mechanisms involving those five miRs. Our results also demonstrated a remarkable decrease in the serum level of hsa-miR-590-5p and a significant increase hsa-miR-660-3p compared to the baseline condition (Figure 2A and 2B), indicating a selective modulatory activity of the diet regimen on these two biochemical regulators. As shown in table 2 *in silico* analysis, considering only DIANA miRPath v3.0 program revealed 43 and 27 genes as validated target for hsa-miR-590-5p and has-miR-660-3p, respectively. Of note, DIANA miRPath v3.0 program, is a web server established for identification of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways corresponding to the networks of miRNA targets by superimposing numerous miRNA-target relationships on the merging and meta-analysis algorithm [32]. Furthermore, recent genome-wide association studies have pointed out that none of genes in table 3 were involved in the common migraine form [33]. Remarkably, inflammatory mediators are elevated in young women with migraine, and these levels correlate with headache frequency and body mass index [34] but biomarkers for migraine stratification and diagnosis still challenge and miRs are good candidate [35,36]. Under the biphasic KD diet the serum levels of has-miR-590-5p and has-miR-660-3p change and this could might contribute to restore brain cortical response. In this concern, clinical efficacy of KD in migraine, normalizes interictal cortical after 1-month, to the baseline interictal deficit of habituation for both sensory modalities [37]. Therefore, the mechanism of action of KD cannot be restricted only to the management of weight loss. The has-miR-590-5p was found increased in the complex regional pain syndrome [38] a condition where migraine assume a risk factor for its development [39]. Amongst the validated target genes found for hsa-miR-660-3p, which is strongly upregulated, there are GRIN2B and SLC38A1 also known as the glutamine transporter SNAT1, its expression is largely restricted to brain or central neurons (Figure 3A-3D). Specifically, it was found in rat cerebral cortex and neighboring structures, with a note on its localization in human cortex [40]. The inhibition of SLC38A1 confers neuroprotection in mice by the modulation of autophagy system [41]. If the major biomechanism of KD in improving clinical and neurophysiological effects of migraine [42] is uniquely based on the modulation of miRs remains to be determined. It is worth to note that body weight was signifi-

cantly reduced (Figure 4) and obesity is sustained by subclinical inflammation state that with the KD could ameliorate.

In conclusion, our study demonstrates that biphasic KD is able to reduce the frequency and intensity of pain attack in migraineurs female obese subjects.

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Bibliography

1. Lipton RB and Bigal ME. "Migraine: epidemiology, impact, and risk factors for progression". *Headache* 45 (2005): S3-S13.
2. Bigal ME., *et al*. "Obesity and migraine: a population study". *Neurology* 66.4 (2006): 545-550.
3. Bigal ME., *et al*. "Body mass index and episodic headaches: a population-based study". *Archives of Internal Medicine* 167.18 (2007): 1964-1970.
4. Ford ES., *et al*. "Body mass index and headaches: findings from a national sample of US adults". *Cephalalgia: An International Journal of Headache* 28.12 (2008): 1270-1276.
5. Peterlin BL., *et al*. "Obesity and migraine: the effect of age, gender and adipose tissue distribution". *Headache* 50.1 (2010): 52-62.
6. Hershey AD., *et al*. "Obesity in the pediatric headache population: a multicenter study". *Headache* 49.2 (2009): 170-177.
7. Kinik ST., *et al*. "Obesity and paediatric migraine". *Cephalalgia: An International Journal of Headache* 30.1 (2010): 105-109.
8. Pinhas-Hamiel O., *et al*. "Headaches in overweight children and adolescents referred to a tertiary-care center in Israel". *Obesity (Silver Spring)* 16.3 (2008): 659-663.
9. Ravid S. "Migraine and paediatric obesity: a plausible link?" *The Indian Journal of Medical Research* 139.3 (2014): 343-348.
10. Verrotti A., *et al*. "Obesity and headache/migraine: the importance of weight reduction through lifestyle modifications". *BioMed Research International* (2014): 420858.
11. Brownlow ML., *et al*. "Nutritional Ketosis Affects Metabolism and Behavior in Sprague-Dawley Rats in Both Control and Chronic Stress Environments". *Frontiers in Molecular Neuroscience* 10 (2017): 129.
12. Brehm BJ., *et al*. "A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women". *The Journal of Clinical Endocrinology and Metabolism* 88.4 (2003): 1617-1623.
13. Gross EC., *et al*. "Potential Protective Mechanisms of Ketone Bodies in Migraine Prevention". *Nutrients* 11.4 (2019).
14. Barbanti P., *et al*. "Ketogenic diet in migraine: rationale, findings and perspectives". *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 38 (2017): 111-115.
15. Nelson ED., *et al*. "Epigenetics in the mature mammalian brain: effects on behavior and synaptic transmission". *Neurobiology of Learning and Memory* 96.1 (2011): 53-60.
16. Eising E., *et al*. "Epigenetic mechanisms in migraine: a promising avenue?" *BMC Medicine* 11 (2013): 26.
17. Hu XM., *et al*. "Downregulation of miR-219 enhances brain-derived neurotrophic factor production in mouse dorsal root ganglia to mediate morphine analgesic tolerance by upregulating CaMKIIgamma". *Molecular Pain* 12 (2016).
18. Ligon CO., *et al*. "Targeting Epigenetic Mechanisms for Chronic Pain: A Valid Approach for the Development of Novel Therapeutics". *The Journal of Pharmacology and Experimental Therapeutics* 357.1 (2016): 84-93.
19. Gallelli L., *et al*. "microRNAs-based predictor factor in patients with migraine-ischemic stroke". *Microrna* (2017).
20. Gallelli L., *et al*. "microRNAs to monitor pain-migraine and drug treatment". *Microrna* (2017).
21. Lagos-Quintana M., *et al*. "Identification of novel genes coding for small expressed RNAs". *Science* 294.5543 (2001): 853-858.
22. Cannataro R., *et al*. "Ketogenic Diet Acts on Body Remodeling and MicroRNAs Expression Profile". *Microrna* 8.2 (2019): 116-126.
23. Cannataro R., *et al*. "Ketogenic Diet and microRNAs Linked to Antioxidant Biochemical Homeostasis". *Antioxidants (Basel)* 8.8 (2019).

24. Cannataro R., *et al*. "Modulation of MicroRNAs Linked to Pain-migraine by Ketogenic Diet". *Current Developments in Nutrition* 3 (2019).

25. Geiss GK., *et al*. "Direct multiplexed measurement of gene expression with color-coded probe pairs". *Nature Biotechnology* 2008 26 (3): 317-325.

26. Waggott D., *et al*. "NanoStringNorm: an extensible R package for the pre-processing of NanoString mRNA and miRNA data". *Bioinformatics* 28.11 (2012): 1546-1548.

27. https://www.nanostring.com/products/analysis-software/nsolver

28. Shkurnikov MY., *et al*. "Analysis of Plasma microRNA Associated with Hemolysis". *Bulletin of Experimental Biology and Medicine* 160.6 (2016): 748-750.

29. Bond DS., *et al*. "Migraine and obesity: epidemiology, possible mechanisms and the potential role of weight loss treatment". *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity* 12.5 (2011): e362-371.

30. Pavlovic JM., *et al*. "Association Between Obesity and Migraine in Women". *Current Pain and Headache Reports* 21.10 (2017): 41.

31. Andersen HH., *et al*. "Serum MicroRNA Signatures in Migraineurs During Attacks and in Pain-Free Periods". *Molecular Neurobiology* 53.3 (2016): 1494-1500.

32. Vlachos IS., *et al*. "DIANA-miRPath v3.0: deciphering microRNA function with experimental support". *Nucleic Acids Research* 43 (2015): W460-466.

33. Klimov E., *et al*. "Genetics of Migraine - Is There any Progress?" *Journal of Neurology Stroke* 7.4 (2017): 00245.

34. Tietjen GE., *et al*. "Adverse childhood experiences are associated with migraine and vascular biomarkers". *Headache* 52.6 (2012): 920-929.

35. Gazerani P. "Current Evidence on Potential Uses of MicroRNA Biomarkers for Migraine: From Diagnosis to Treatment". *Molecular Diagnosis and Therapy* 23.6 (2019): 681-694.

36. Gallelli L., *et al*. "Hsa-miR-34a-5p and hsa-miR-375 as Biomarkers for Monitoring the Effects of Drug Treatment for Migraine Pain in Children and Adolescents: A Pilot Study". *Journal of Clinical Medicine* 8.7 (2019).

37. Di Lorenzo C., *et al*. "A ketogenic diet normalizes interictal cortical but not subcortical responsivity in migraineurs". *BMC Neurology* 19.1 (2019): 136.

38. Orlova IA., *et al*. "MicroRNA modulation in complex regional pain syndrome". *Journal of Translational Medicine* 9 (2011): 195.

39. Peterlin BL., *et al*. "Migraine may be a risk factor for the development of complex regional pain syndrome". *Cephalalgia: An International Journal of Headache* 30.2 (2010): 214-223.

40. Melone M., *et al*. "Localization of the glutamine transporter SNAT1 in rat cerebral cortex and neighboring structures, with a note on its localization in human cortex". *Cereb Cortex* 14.5 (2004): 562-574.

41. Yamada D., *et al*. "Inhibition of the glutamine transporter SNAT1 confers neuroprotection in mice by modulating the mTOR-autophagy system". *Communications biology* 2.1 (2019): 346.

42. Goadsby PJ., *et al*. "Pathophysiology of Migraine: A Disorder of Sensory Processing". *Physiological Reviews* 97.2 (2017): 553-622.