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FUNCTIONAL GENOMICS: MICRO RNA IN LIPID METABOLISM AND OBESITY

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ABSTRACT
Obesity has become a pandemic afflicting global population, irrespective of socio-economic status. Considered the master disorder, culminates in Type 2 Diabetes Mellitus (T2DM), Non-alcoholic Fatty liver disease (NAFLD), Non-alcoholic Steatohepatitis (NASH), hypertension and several cardiovascular diseases (CVDs). Dietary lipids contribute significantly to diet-induced obesity therefore, lipid metabolism requires regulation at the cellular level by intra-cellular regulatory networks from the influence of intrinsic and environmental factors on metabolic homeostasis, which is essential for physiological wellbeing. Increased levels of low density lipoproteins (LDLs), Cholesterol and Triglycerides (TGs) alters lipid levels thereby altering tissue-specific gene expression in human pathologies. Aberrant lipid metabolism, dysregulated Sterol Regulatory Element Binding Proteins (SREBPs) and Liver X receptors (LXRs) were so far cited as major reasons attributing to obesity. Recently, regulation of cholesterol and fatty acid metabolism by Small non coding RNAs (SncRNAs) such as Micro RNAs (miRNAs) were confirmed in worms, rodents and mammalian models. A long list of miRNAs with tissue specific expression (miR-33 family, miR-27, miR-122, miR-370, miR-335, miR-143, miR-378 and miR-125 family) had been suggested to play a crucial role in lipid metabolism. Our understanding of the molecular mechanism of post-transcriptional regulation of cellular homeostasis and functional genomics of miRNAs in regulation of genes involved in non-communicable metabolic disorders like obesity, NAFLD, non-alcoholic steato-hepatosis (NASH) and Hepato-Cellular Carcinoma (HCC) remains obscure. This review addresses post-transcriptional regulation of lipid metabolism by miRNAs, key targets and beneficial applications for developing targeted therapeutics in humans.

KEYWORDS: obesity, dietary lipids, SncRNAs, miRNAs, lipid metabolism, SREBPs, post-transcriptional regulation, metabolic homeostasis, gene silencing.

INTRODUCTION
Prevalence of obesity has become threefold and continues to grow at an alarming pace causing grave health issues among populations in developed and developing nations, alike.[1,2,3] Obesity is a non-communicable, multifactorial metabolic disorder characterized by accumulation of white adipose tissue (WAT) in sub-cutaneous, intra-abdominal and vascular organs in vertebrates, including zebrafish.[4, 5, 6, 7] More than 2/3 of the adults in USA is obese and ~60% in the UK are overweight with India ranking next only to USA and China.[8, 9] Obesity is a major risk factor for several cardiovascular diseases (CVDs), the leading cause of mortality affecting young adolescents. Hence, prevention and obesity management has become expensive over the years, causing a great concern for developing nations.[4, 10, 11] India has become one of the capitals of diabetes and cardiovascular diseases especially, with more cases reported in young adolescents from southern India.[12, 3] National Family Health Survey (NFHS) noted a steady rise in diet-induced obesity from 11% (NFHS2) to 15% (NFHS3) among Indian women aged 15-49 years, demanding immediate attention from government and NGO organizations.[10] Genetic obesity in humans occur by mutations in obesogenic genes with more inter-individual variations among different populations.[5] Diet-induced obesity is primarily caused by excess consumption of cholesterol rich diet, altered gut microbiome composition, sedentary life style and aberrant cholesterol metabolism leading to altered expression of genes involved in lipid metabolism and CVDs.[13,14,15] Increasing levels of low density lipoprotein (LDL) accelerates the risk of atherosclerosis and coronary heart diseases (CHDs) but high density lipoproteins (HDLs) reverses the effect in obese mouse and humans.[6, 16-18] Excessive deposition of triglycerides

in liver may also lead to co-morbidities like non-alcoholic fatty liver disease (NAFLD), non-alcoholic hepatic steatosis (NASH), fibrosis and/or Hepatocellular Carcinoma (HCC) upon prolonged consumption of high cholesterol diet (HCD) and delayed regeneration of organs or even cancer in humans.[17, 19-23]

Genetic Predisposition and GWAS Studies

Environmental factors such as shift working, insufficient sleep, endocrine disruptors, smoking and anti-depressants induces epigenetic modifications that may also augment central or peripheral obesity induction by inducing circadian asynchrony in coordination with altered physiological pathways.[2, 18, 24-26] Surprisingly, circadian asynchrony also alters expression of CLOCK genes and downstream tissue-specific Clock Controlled Genes (CCGs) involved in metabolism leading to chronic disorders like NAFLD, NASH and Hepatocellular carcinoma (HCC) in rodents and humans.[19, 21, 23, 27- 29] Only ~12% of single-nucleotide polymorphisms (SNPs) responsible for obese phenotype has been identified by Genome Wide Association Studies (GWAS), remaining SNPs lie in non-coding or inter-genic regions within the human genome are under the direct miRNA regulatory control mechanism, that are currently under investigation.[5, 30] Presently, GWAS had gained much attention owing to their pace of gene identification using 2 genetic epidemiological approaches namely candidate genes and genome wide linkage studies responsible for obesity. However, obesity is a multifactorial disorder an outcome of multiple genetic and extrinsic factors augmenting the risk of obesity in genetically prone humans.[31] Genome wide linkage studies are hypothesis generating, but whole genome GWAS identifies previously unsuspected genetic loci involved in polygenic disease like obesity in global population.[32, 33] Studies have uncovered >18 novel genetic loci present in persons susceptible to obesity; allelic variants and mutations in genes like melanocortin 4 receptor (*MC4R*), β Adrenergic Receptor B3 (*ADRB3*) and pro-hormone convertase 1/3 (*PCSK1*) results in obesity and are considered promising candidates as reliable biomarkers in children and may aid in determining obesogenic gene expression in persons susceptible to such metabolic disorders.[30-34] Our understanding of the biology and pathophysiology of mechanisms underlying onset, development and regulation of obesity in humans at genome level, is limited. Genetic predisposition to obesity by genetic mutations and polymorphisms in genes regulating metabolism especially by genome wide methylation is well known to increase appetite by constitutively activating *akt/agrp* overexpression responsible for controlling appetite and metabolism in humans and zebrafish.[35, 36] Further, altered adiponectin, ghrelin and leptin expression by epigenetic modifications or mutations in regulating transcription factors like Sterol Regulatory Element Binding Proteins (SREBPs) and Peroxisome Proliferator Activated Receptors (PPARs)[37, 38] can be identified at an early stage from umbilical cord tissues of infants by perinatal epigenetic analyses using Sequenom Mass ARRAY.[5, 32, 38, 39] While, SREBP1 isoforms regulate fatty acid metabolism, SREBP2 is the master regulator of cholesterol metabolism. Most authors focussed on canonical transcriptional regulatory pathways, while novel mechanisms by which lipogenic genes were post-transcriptionally regulated remained a mystery until miRNAs were reported to regulate gene expression post transcriptionally during early embryonic development in the nematode *Caeonrabhditis elegans.*[40-43]

Bartel (2009)[44] reported Micro RNAs (miRNAs) in humans are tiny but mighty regulators that targets genes underlying lipid homeostasis and metabolic disorders. Altered miRNA expression pattern in response to genetic or environmental factors lead to aberrant gene expression contributing to abnormal organ functioning and pathology in humans.[45, 46] A long list of miRNAs have been compiled (MicroRNA.org) known to modulate gene expression in lipid metabolism **(Fig. 1)** in response to altered intrinsic and extrinsic/environmental factors at the cellular level.[45] Tissue specific miRNA signatures have been documented in several metabolic disorders.[45-50] Interestingly, miRNAs can have a modest effect on single or multiple mRNAs showing potent post-transcriptional regulatory mechanism hence, hundreds can be targeted by a single miRNA thereby increasing repression of mRNAs involved in altered metabolic pathways. They can also serve as unique biomarkers and potential targets to be successfully utilized in targeted therapeutics to treat human disorders.[20, 23, 51-53] Frequently, miRNAs are also known to self-regulate by a feed-forward and feedback mechanism thereby regulating their own expression in tissues (liver) involved in lipid and metabolic homeostasis.[20,44,51] Thus, miRNAs have finally emerged as key players but their functional role in cellular maintenance and metabolic homeostasis remains obscure.[44,47] For better understanding the functional role of miRNAs, we have briefly described their biogenesis and mechanism of action.

MicroRNA biogenesis and gene silencing mechanism

Micro RNAs (miRNAs) are Short non-coding RNAs (SncRNAs) that post-transcriptionally regulate gene expression, reported in early embryonic development to complex patho-physiological and metabolic disorders in humans.[40- 43, 47] Lee et al. (1993)[40] and Ambros (2003)[42] demonstrated in the nematode, *C. elegans*, the novel mechanism by which miRNAs are generated and their ability to target multiple mRNAs for gene silencing during embryonic development, highlighting their ability to post-transcriptionally modulate multiple gene expression in specific tissues.[40-44] Micro RNAs are single stranded, short (~22 nucleotides) non-coding RNAs transcribed by RNA Polymerase II (Pol II) as precursor RNAs (pre-miRNAs) from either inter-genic, intronic or polycistronic genomic loci. Micro RNAs are processed by both canonical and non-canonical pathways.[43] Canonically, miRNAs are processed by

Drosha and DGCR8 RNAse complex or splicesome within the nucleus. Precursor RNAs are further processed by Drosha and DGCR8 RNAse III complex and trimmed to become pre-miRNA in the nucleus. However, non-canonically, miRNAs are transcribed directly from the genome as endogenous short hairpin RNAs (endo-shRNAs) or by direct splicing of introns resulting in hairpins termed "mirtrons".[42-44] Subsequently, double stranded pre-miRNAs derived from both canonical and non-canonical pathways are exported into the cytoplasm through nuclear pores by an exportin 5-RAN GTP complex, where it gets cleaved to become miRNA duplex. Processing by Dicer and transactivation response RNA binding protein (TRBP) and RNAse III transforms double stranded pre-miRNAs duplex from native stem loop structure into a mature 22mer hairpin looped miRNA, prepared to target multiple mRNAs.[41- 43]

Targeting of mRNAs occurs after undergoing modifications in the RNA-Induced Silencing Complex (RISC). Argonaute proteins help in incorporation of the miRNA targeting strand (guide strand) forming a RNA-induced silencing complex (RISC) which directs them to the specific cellular targets.[44] Initial recognition of mRNAs occur by simple Watson-Crick base pairing of the seed sequence (2-8 nucleotides) with the mRNAs 3' UTRs and later extended by additional pairing at 5' ends which results in repression of mRNA expression or degradation.[40-44] Briefly, guide strand in miRNA identifies and targets 3' untranslated regions (UTRs) of a single or multiple mRNAs with the help of 'seed-sequence' and pairs along with RISC complex leading to destabilization and repression of mRNA expression (Fig. 1).[42, 44, 54] Ability to target single or multiple mRNAs is determined by specific 'seed sequence' in mRNAs that determines the target of miRNAs, thus regulating expression of single or multiple genes in metabolic or molecular pathways. Interestingly, seed sequence and miRNAs remain conserved from lower organisms to the mammals.[42,45-48] Though, plants follow similar biogenesis pathway, major difference between plant and animal miRNAs is the lack of complete complementarity between miRNA and mRNA sequences in animals. In plants, complete complementarity has been reported to promote mRNA degradation, despite such significance of partial or complete complementarity is related to translational repression by mRNA de-adenylation and or degradation or sequestration into P-bodies, respectively.[43] This review addresses recent advances made in the field of miRNA mediated regulation of lipid metabolism through established pathways in non-communicable metabolic disorders like obesity. We will also discuss the impact of such post-transcriptional regulation gene silencing mechanism on health and disease in humans, their potential to serve as therapeutic targets and ways to unravel the underlying pathophysiology for effective utilization of the identified novel miRNAs and target genes in obesity.

Functional regulation of lipid metabolism

Lipid homeostasis is an important aspect of cellular and molecular physiology, alterations of which leads to physiological disorders. Lipid metabolism involves both cholesterol, fatty acid synthesis and absorption and β oxidation in hepatocytes which is tightly regulated by regulatory factors.[5, 47] Several transcription factors (TFs) are known to regulate lipid homeostasis in humans, the most important being the Sterol Regulatory Element Binding Proteins, SREBP class of TFs which directly or indirectly coordinate the expression of majority of the lipogenic genes involved in fatty acid synthesis, uptake and metabolism.[7,14,39,55] Interestingly, cholesterol homeostasis in mammals is also regulated by white or brown adipocyte tissues (WAT/BAT).[56,57] Membrane bound SREBPs possess two homologues (SREBF1and SREBF2) of which, two SREBF1 isoforms (SREBP1a & 1c) serve as the major regulator of most lipogenic genes while Peroxisome Proliferator Activated Receptors (PPARs) act on cholesterol synthesis pathways.[38, 39, 47, 55, 58] A host of genes were identified to be involved in monogenic, syndromic and polygenic obesity in humans.[1,5,45] Earlier, in obesity, regulation of cholesterol, fatty acid biosynthesis and metabolism was thought to be mediated solely by SREBPs, under feedback control.[16, 39, 55, 58] however, recent studies have confirmed the potential role of other homologues of sterol sensing membrane bound SREBF1/SREBP1a & SREBP1c and SREBF1, Peroxisome Proliferator Activated Receptors (PPARs) and Liver X Receptors (LXRs) on *de novo* lipid synthesis in obesity.[7, 38, 39] For detailed information on the genesis and functional significance of SREBPs, readers are directed to other reviews.[16, 39, 55] Over the years, data has accrued on the contribution of numerous transcription factors and nuclear receptors in regulating lipid homeostasis in mouse and humans but, regulation of lipogenic genes and metabolism in chronic diseases like NAFLD, NASH and HCC remains unclear.[7, 20, 23, 59, 61]

Very recently, role of miRNAs (miR-122, miR-33, miR-143) in controlling cholesterol homeostasis by affecting fatty acid and cholesterol metabolism in rodent hepatocytes was reported.[62,63,64] A sharp increase in the levels of cholesterol, LDLs, HDLs, TGs and alanine aminotransferase leading to NAFLD had been reported in HCD fed mice.[19, 20, 23] Phospholipids, triglycerides and cholesterol regulation using antagomirs was also confirmed by reduced plasma cholesterol levels in mice.[62- 64] However, very few authors have tracked the physiology of lipid absorption *in vivo* and genes involved using Engineered Fluorescent Lipid Probes (EFLPs). Zebrafish with genomic similarity to humans have *fat free* (*ffr*) mutants that failed to survive after few days post hatching owing to abnormal lipid metabolism due to biliary defects resulting in aberrant cholesterol transport.[60] Later, Justin et al. (2010)[6] showcased fluorescent *in vivo* screening using EFLPs, that drugs like statins (simvastatin, atorvastatin) and Ezetimibe prescribed for cardiovascular patients efficiently

inhibited *de novo* cholesterol and fatty acid synthesis and absorption by inhibiting HMG-CoA reductase activity and NPC1L1 mediated cholesterol transport in zebrafish larvae and adults, similar to humans suggesting researchers may be able to uncover molecular targets underlying non-communicable, patho-physiological disorders, using zebrafish models.[6, 7, 15, 17, 21, 36, 60]

Micro RNAs are depicted as key players in orchestrating gene expression in cholesterol, fatty acids, triglyceride metabolism.[23,41,42] Functional role of miRNAs in cholesterol synthesis and lipid absorption, more importantly in regulation of obesity are still under investigation.[28-30, 45-47] Many miRNAs well known to be involved in lipid metabolism were recently reported i.e. miR-122, miR-143, miR-370, miR-378/378*, miR-335, miR-125a- 5p and miR-33 etc. of which miR-122 and miR33a are known to be associated with hepatocytes from studies in mouse models but their functional significance remains at large.[45, 62-64] Interestingly, hepatic miRNAs such as miR-122 and miR-33 family forms the majority of the total miRNA expression in the liver, which coordinates with miR-370, miR-125a-5p and miR-378/378*.[20, 62, 63, 64] Micro RNA 122 are one among the highly regulated members of the β-oxidation pathway.[65, 66] Rayner et al. (2010)[67] investigated the role of miR-33a and miR-33b family, set of intronic miRNAs transcribed from the same genetic loci coding for SREBF1 in primates in regulating cholesterol homeostasis. Since, they arise from intron 16 of human SREBPF-2 and plays significant role in cholesterol export through regulation of *ABCA1*, *NPC1* and *ABCG1* genes hence, miR-33a is rightly considered the master regulator of cholesterol metabolism.[67] Horie *et al.* (2014)[68] gave experimental evidence to confirm that miR-122, a liver-specific miRNA and miR-33a and 33b forms a regulatory circuit with SREBF1 in maintaining lipid homeostasis by reducing HDL-C through *in vivo* studies using knock in mouse models (Figure 1). Presently, the above miRNAs are being considered as potential therapeutic targets for treating metabolic disorders including obesity and CVDs using genetically modified mouse and zebrafish models.[67-69]

Functional regulation of fatty acid metabolism

There are several miRNAs known to target fatty acid metabolism in the liver.[61] Despite their small size, miRNAs play a crucial role in regulating expression of major genes involved in fatty acid metabolism in the liver by co-ordinating with carnitine palmitoyl transferase (*Cpt1a*), Carnitine O-Octanoyl transferase1 (*Crot*1) and Hydroxy acyl-CoA Dehydrogenase (*Hadhb*).[66, 68] CROT1 protein helps in assembling short chain fatty acids to carnitine for transport into mitochondria and HADHB, required for final steps in β oxidation.[70] Binding sites for miR-33 is well conserved in the 3' UTRs of *Cpt1a*, *Crot1* and *Hadhb* in which CPT1a protein is essential for medium and long chain fatty acids transport to mitochondria.[67] Role of miR-33 in targeting each of these genes by different mechanisms were less investigated. Unexpectedly, overexpression of miR-33 lead to down regulation of CPT1 and HADHB and indirectly reduced β-oxidation in heaptocytes.[65] Though, miR-33a and miR-33b differs only by 2 nucleotides, both targets fatty acid β oxidation pathways by regulating expression of ATP-Binding Cassette Transporter (ABCA1) and AMP- activated Protein Kinase *α* (*Ampkα*) thus targeting critical steps involved in fatty acid β-oxidation.[66] In addition to regulating fatty acid degradation, miR-33a and miR-33b also regulate crucial upstream regulators of lipid homeostasis and ageing.[71] Recent studies confirmed that SIR-2 Protein 6/Sirtuin-6 (SIRT6), a histone deacetylase and a key player in glucose metabolism that confers resistance to oxidative stress is under the direct control of miR33a and miR33b.[71] Further, research is in progress to evaluate the potential of using miR-33 family antagomirs upon SIRT6 expression as therapeutic targets against ageing in humans.[72]

Functional regulation of hepatic lipid metabolism

Researchers have elucidated the functional role of miRNAs in energy homeostasis in metabolic disorders. Several miRNAs, well known to regulate lipid metabolism especially miR-122, miR-370, miR-335, miR125a–5p, miRNA378/378*, miR-33a-33b and miR-122 have been documented to exhibit tissue specific expression in mouse and humans.[62-68, 72, 73] Of which, miR-122 accounts for >70% of the total liver miRNA expression and plays a significant role by altering expression of hundreds of genes in NAFLD and NASH.[19, 50-53] Down regulation of liver specific expression of miR-122 using antagomirs altered expression of specific genes (CROT1, HADHB, CPT1a etc.) in cholesterol bio-synthetic pathway (Fig. 1), fatty acid synthesis and β oxidation, affecting metabolic homeostasis in mice.[62,72] Antagomirs induced silencing of miRNA 122 also protected high-fat fed mice against hepatic steatosis and fibrosis by reducing cholesterol and fatty-acid synthesis and metabolism.[19, 20, 49, 64, 66] Further, miR370 targeting of *Cpt 1a*, a mitochondrial enzyme that mediates fatty acid β oxidation negatively upregulated expression of miR-122, thereby indirectly increasing SREBP1c and Diacyl Glycerol O-Acyltransferase-2 (DGAT2) expression.[64-66] Surprisingly, miR-378/378* were shown to regulate lipid metabolism by a peculiar mechanism; miRNA arises from intronic region of *PPARγ* coactivator-1 alpha (*PGC1α*).[73] Overexpression of miR-378/378* not only increased triacylglycerol production and accumulation, but also enhanced expression of genes involved in fatty acid metabolism i.e. fatty acid binding protein 4 (FABP4), fatty acid synthase (FAS) and stearoyl coenzyme A desaturase (SCD-1) in the β-oxidation pathway.[62, 65, 73] Also, miR-27 and miR-335 were found to be associated with adipocyte differentiation. While, miR-27 inhibits expression of PPARγ, C/EBP*α* gets down regulated, antagonistically upregulating miR-335 in liver and adipose tissues of obese mice.[63,74] Hence, functional significance and mechanism of miR-335

upregulation in adipogenesis are currently being investigated. Expectedly, researchers are now considering miR-122, miR335, miR378/378* and miR27 as attractive therapeutic targets for treating adipogenesis, hepatic steatosis and cirrhosis.[19, 20, 23, 62, 63, 68, 72]

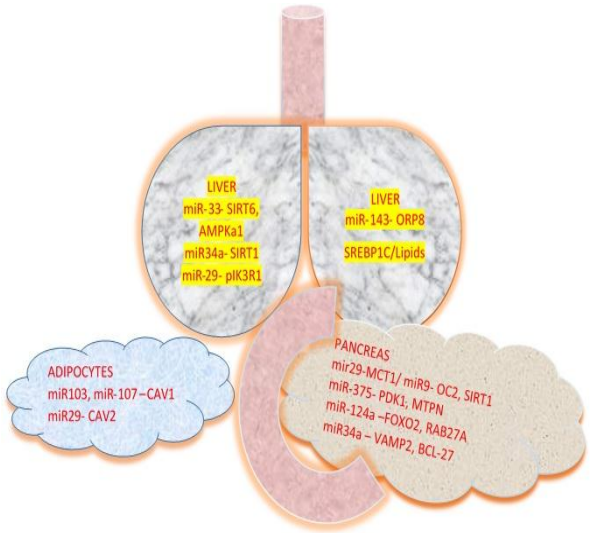

Figure1. Micro RNAs involved in Lipid and cholesterol metabolism.

Nieman Pick C1 (NPC1) are novel trans-membrane protein that play an essential role in cellular cholesterol transport in vertebrates and inhibited by the drug Ezetimibe, *Zetia.*[6, 75, 76] Of the total load, 50% comes from intestinal cholesterol of which two thirds is from bile and one third through dietary lipids, which requires a transporter protein.[77] NPC1-mRNA have two binding sites in their 3' UTRs for miR-33 which inhibit their expression while, mouse has only one site resulting in only modest inhibition of NPC1 protein expression thereby impairing cholesterol transport.[47, 69,77] Dietary cholesterol modulates expression of miR-33b and its isoform miR-33a.[67] Although, a key-post transcriptional regulator of cholesterol homeostasis, miR-33 are upregulated only in response to cholesterol depletion in cells, as confirmed by their temporal expression.[78] Interestingly, *in vivo* inhibition of miR-33 lead to overexpression of liver ABCA1 protein thereby increasing levels of HDLs, confirming their inverse control over major transporter proteins like ABCA1 and ABCG1.[67,78] Increasing high density lipoproteins (HDL) is beneficial in cardiovascular diseases hence, therapeutic approaches to increase HDL levels by altering miR-33 expression in cardiovascular patients would be fruitful in reversing the pathology.[6,67,72] In mouse models, miR-33 also inhibits ABCG1 expression affecting cholesterol efflux but not in humans owing to the absence of conserved 3' UTRs in human mRNA.[78] Therapeutic approaches utilizing miR-33 to inhibit ABCA1 or ABCG1 proteins in obese patients may lead to reduction in bad cholesterol levels and prevent cardiovascular insults and help cardiac regeneration.[65-68] Mechanism and molecular targets of miRNAs, their *in vivo* regulation by feedback and feed forward mechanism in lipid metabolism and associated co-morbidities may also be efficiently investigated *in vivo* using inexpensive genetic models like zebrafish.[15, 7, 36]

Functional regulation of obesogenic gene expression

Nutritional excess, altered sedentary lifestyle, lack of physical activity and excessive consumption of fatty rich diets, energy expenditure and circadian asynchrony induced by shift working were considered the major reasons augmenting adiposity in mammals and manifesting in severe co-morbidities.[21, 24-27, 68] Role of miR-143, miR-204, miR-141, miR-200a, miR-200b, miR-200c and miR-429 has been implicated in early adipogenesis i.e. differentiation of adipocytes thus regulating the complex network in numerous ways[79, 80]; miR-143 is also known to regulate Extra cellular signal Regulated Kinase 5 (*ERK5*) and Mitogen Activated Protein Kinase7 (*MAPK7*) during *in vitro* adipocyte differentiation while miR-204, miR-141, miR-200a, b, c and miR-429 are known to regulate adipocyte cell fate determination in animal models[63-67] (Table 1). While, normal adipogenesis is regulated by transcriptional factors such as *PPARγ* and its co-activators *PGC1α* and the family of CCAAT/Enhancer Binding Protein (*C/EBP*) and Kruppel like factors (*KLF*s) during formation of white adipose tissue i.e. adipogenesis.[62, 80, 81] Well-known miRNA clusters such as miR-27a, b, c/ miR-17, miR-92, miR-130, miR-378* were reported in terminal differentiation and maturation of White Adipose Tissue (WAT)[74,81] while, miR-193b and miR-365 play a major role in BAT differentiation by repressing myogenesis.[57] Harnessing miRNAs expression with antagomirs and generating knock in/out models may help develop therapeutics for human obesity and co-morbidities.

Table 1. Functional role of MicroRNAs (miRNAs): Major miRNAs involved in lipid metabolism and targets in human metabolic disorders

Micro RNAs	Target genes	Functional role	Visceral organs involved
miR-122	SLC7A1 (CAT1), ADAM17	Lipid metabolism	Liver
miR-143	ERK5 (MAPK7)	Adipocyte differentiation, insulin resistance	Adipose, Liver and Pancreas
miR-33a family (a,b)	ABCA1, NPC1, CPT1A, HADH8, CROT, SIRT6	Lipid and Cholesterol Homeostasis	Liver, Intestine

miR-34a	SIRT1, VAMP2	Lipid metabolism, B Cell exocytosis	Liver, Pancreas
miR-378 and miR-378*	ESSRG, GABPA	Adipocyte differentiation, lipid synthesis	Adipose
miR-130	PPARG	Adipogenesis	Adipose tissues
Let-7	IGF1R, INSR, IRS2, HMGA2	Insulin sensitivity	Muscle, Adipose

Abbreviations used: SLC7A1: solute carrier 7A1; VAMP2 – VESICLE ASSOICATED MEMBRANE PROTEIN 2; HADH8- 3-hydroxyacyl CoA dehydrogenase; CROT-CARNITINE O OCTONOYL TRANSFERASE; SIRT6- SIR-2 Protein 6; PPARG-peroxisome proliferator activated-receptor; IGF1R-Insulin like growth factor 1; INSR- Insulin receptor; IRS2- Insulin receptor substrate 2; HMGA2- high-mobility group protein A2; CAT1- cationic amino acid transporter 1; ADAM17- ADAM metallo-peptidase domain 17; ERK5/MAPK7- extra-ceullar signal regulated kinase; ABCA1- ATP binding cassette subfamily A member1; NPC1- Niemann-PickC1 protein; CPT1A- Carnitine palmitoyl transferase 1A.

Although, mutation in genes are responsible for inducing genetic obesity in ~40-70% obesity in humans.[5] Only few studies have documented the impact of mutations as major reasons leading to obesity associated alterations in miRNA expression in WAT and BAT differentiation[81]; critical evidences remain unearthed and their role in inducing adipogenesis and obesity yet remains to be understood[62, 74, 80] Except for the GWAS studies, all the above results have arisen from *in vitro* studies rather than *in vivo.* Hence, detailed studies on the regulatory role of individual miRNAs on genes responsible for genetic obesity and energy homeostasis using in expensive animal models like zebrafish is the need of the hour which may provide a robust platform for rapid drug discovery.[21, 36, 81]

Functional regulation of lipid metabolism in zebrafish

Zebrafish has become an international vertebrate model for studying molecular mechanisms underlying several human metabolic disorders including obesity, T2DM, NAFLD, cardiovascular diseases and cancer owing to their genomic and organ similarity.[6, 7, 18, 35, 36, 60, 82, 83] Though, miRNAs were first reported in the nematode, *C. elegans* as regulators of embryonic development subsequently, several studies from plants, lower animals and humans confirmed their presence and role in gene silencing by post-transcriptional regulation.[40-44, 36, 50, 51, 84] Rottiers and Naar (2012) and several others[46-48] have showed that miRNAs indeed play a crucial role in regulating dietary lipid metabolism in human metabolic disorders, affecting multiple organs and physiological pathways through interconnected regulatory networks. Most importantly, in all vertebrates, miRNAs play a lead role in cholesterol homeostasis in coordination with the Sterol Regulatory Element Binding Proteins (SREBPs) in regulating the lipogenic circuit.[38, 39, 55, 58] Very few miRNAs have been identified in zebrafish to modulate lipid metabolism but their mechanism in metabolic disorders remains hitherto unknown.[21, 22, 36, 85] Since, limited information is available on the molecular and metabolic pathways that miRNAs targets[86] as zebrafish have multiple copies (homologs and orthologs) of same genes due to teleost genome duplication (TGD), an event resulting in multiple homologues.[83] For a detailed list of miRNAs involved in various physiological pathways cloned from zebrafish, readers are directed to related works by other eminent scientists.[86-89]

In zebrafish, lipids through diet or by *de novo* synthesis enters the digestive system, gets converted into LDLs, HDLs and Triglycerides (TGs), transported to liver wherein β oxidation of fatty acids occur in mitochondria which can be monitored *in vivo* using fluorescent lipid probes.[6, 60] Excessive fat are stored as adipose, an essential energy reserve that also serve as an endocrine organ secreting adipokines and cytokines in zebrafish, similar to humans.[7, 21, 36] As in humans, biosynthesis of cholesterol is tightly regulated by SREBPs and LXRs in zebrafish liver by feedback mechanisms.[16, 19, 58, 59, 61] While, circulating levels of LDLs increases the risk of atherosclerosis, excess TGs accumulation in hepatocytes leads to NAFLD ultimately leading to non-alcoholic steato-hepatosis (NASH), liver fibrosis and hepatocellular carcinoma (HCC) in zebrafish.[7, 18-20, 36, 89, 90] Therefore, studies on regulation of cellular lipid homeostasis by miRNAs in metabolic disorders and cancer using humanized zebrafish disease models are underway.[7, 21, 36, 82, 83, 90] Small RNA profiling of zebrafish cell lines and organs by cloning small non coding RNAs (SncRNAs) confirmed functional homology with humans in physiological pathways in obesity and related comorbidities.[50-52, 85-89] So far ~ 400 plus miRNAs had been reported in zebrafish and continues to grow at a rapid pace after the whole genome sequence was published.[85, 89] Many miRNAS showed organ specific expression pattern during initial and later stages of development in zebrafish.[88-91] Recently, Kloosterman et al. (2006)[89] reported 66 new miRNAs in addition to the previously known miRNAS (~150), suggested to play a potential role in early embryonic, larval development and digestive physiology increasing the total to about 87 distinct families in zebrafish[85]; removal or silencing of all miRNAs using antagomirs resulted in developmental arrest in mouse and zebrafish embryos, indicating their functional significance in regulating multiple gene expression.[88, 89, 92]

Zebrafish offers multiple advantages as an *in vivo* system owing to its small size, externally fertilizing embryos that remain amenable to several genetic manipulation and genome editing techniques such as Transcription

Activator like Effector Nucleases (TALENs), Locked Nucleic Acids (LNAs) and Clustered Regularly Interspersed Short Palindromic Repeats(CRISPR) and CRISPR-associated Nucleases (CRISPR-cas) to effectively knock down or edit the gene of interest to bring desirable effects.[36, 83, 85, 92] Transparent zebrafish embryos and larvae also facilitates live tracking of digestive physiology and visceral organ functioning using EFLPs[7,60], within 3 days post hatching (dph) and the availability of complete genome sequence with striking similarity (73%) to humans makes them an attractive humanized genetic model[82, 93, 94, 95] and for investigating dietary cholesterol metabolism, gut microbiome and metagenomics[6, 7, 15, 18, 96] and miRNA regulation of metabolic homeostasis.[85,87-91-96] Concisely, till date, researchers have uncovered >350 miRNA encoding genes that codes for ~200 different mature miRNAs in zebrafish.[91] Despite recent advancements, very few studies have successfully utilized zebrafish for elucidating miRNA regulation of metabolic homeostasis, molecular pathways and key factors involved in CVDs and heart regeneration.[3,21, 22, 60, 85, 94] RNA Deep sequencing of obese zebrafish model during heart regeneration revealed unique miRNA signatures representing the conserved regeneration machinery among the vertebrates.[22, 89]

Researchers have highlighted crucial role of miRNAs in post-transcriptional gene silencing during embryonic development and metabolic disorders since, defective miRNA processing also lead to arrested embryonic development in zebrafish.[85, 88] Further, miRNAs play an important role in zebrafish organogenesis and their temporal expression resembles human visceral organ formation and differentiation.[87] Since, identification of complete set of miRNAs in a model system is crucial for a better understanding of pathophysiology, we are presently using NGS techniques to identify and unravel the miRNA signatures and their functional significance in obesity, heart regeneration, NAFLD and HCC.[7, 19, 22, 50, 53] Currently, in zebrafish, there are few reports on miRNAs associated with obesogenic genes and cholesterol biosynthetic pathways and even less on miRNA regulation of lipid metabolism in obesity.[6, 7, 15, 18] Thus, zebrafish can faithfully serve as potential genetic model for dissecting the molecular targets in metabolic disorders only if functional genomics of miRNA families in different tissues were elucidated using computational methods, beginning from early embryonic development to organ functioning in adults.[17, 19, 23, 28] Transcriptome profiling and RNA deep sequencing using Next Generation Sequencing (NGS) technologies may yield in depth understanding of post-transcriptional gene silencing mechanisms and regulation of genes in metabolic disorders in humans hence, require liberal funding support from national and international funding agencies.[6, 7, 18, 22, 25, 23, 35] Alternatively, replacing the defective copy of an allele in a disorder using CRISPR-Cas and ploidy manipulation techniques in fishes are also promising avenues to explore for therapeutics. In future, studies may focus on effective utilization of miRNAs as therapeutic molecular targets to ascertain speedy recovery from pathophysiological disorders in humans.[36, 51, 89, 94]

Regulation of Lipid Metabolism by SREBPs and LXRs

Cholesterol are essential for cellular growth and remains necessary throughout its life cycle forming a major component of the cell membrane which requires a trans membrane transporter protein NPC1 in humans.[76, 77] Fatty acid synthesis (lipogenesis), is under the control of insulin and carbohydrate levels, while, SREBPs, the membrane bound transcription proteins highly regulate the lipid synthetic machinery, responsible for cholesterol and fatty acid synthesis and homeostasis.[16, 39, 55, 77] SREBPs require a 2 step proteolytic cleavage for entering the nucleus after releasing their amino-terminal bHLH containing nuclear domain, after which they activate wide range of target genes.[38, 55] The nuclear SREBP after entering the nucleus activates transcription of lipogenic genes involved in cholesterol and fatty acid synthesis by binding to Sterol Regulatory Elements (SREs) or E-boxes by recognizing their palindromic sequence in the promoter region.[39, 58, 61] SCAP and SREBP form a complex on the rough endoplasmic reticulum (ER) membrane. Notably, consensus sequence in SCAP protein for sterol sensing domain is shared by HMG-CoA reductase and Niemann Pick C1 gene product hence, serves as an effective sterol sensor, a prerequisite for SREBP function.[7, 16, 55, 58, 76, 77] Earlier studies have pointed out that controlled regulation of lipogenic gene expression by SREBPS and LXRs happen after sensing sterol deprivation in the cell.[18, 38, 59] In mammals, 3 isoforms of SREBPs (*SREBP 1a, 1c* and *SREBP-2*) regulates expression of >30 genes involved in cholesterol and fatty acid synthesis.[61, 78] Isoform variation and tissue-specific expression of SREBPs have been variably reported from mostly rodent models and cell lines by several authors.[16, 39, 55, 58, 61, 68] Till date, there are 3 SREBPS reported as being coded by two genes, of which SREBP1a and SREBP1c are transcribed from a single gene with alternate promoters while SREBP2 from a different gene[39]; *SREBP-1* is involved in fatty acid metabolism, *SREBP-2* in cholesterol homeostasis.[68] Dysregulation of *SREBP* expression results in diverse physiological and pathological disorders [55, 61]. Cholesterogenic genes usually carry Sterol Response Elements (SREs) in their promoter to which SREBP binds, but varies with different lipogenic genes.[69] Recently, LXRs, another master transcriptional regulators of cholesterol metabolism forming heterodimers with retinoid X receptors (RXRs) and up regulating gene expression in *de novo* cholesterol synthesis has been identified.[16, 38, 59] Briefly, LXRs indirectly regulate transcription of several genes (*Abca1* and *Abcg1*) involved in efflux of cholesterol and therefore, mutation in LXRs result in atherosclerosis and also accumulation of sterols in liver leading to hepatic steatosis and fibrosis[19, 20, 59] but when targeted by pharmacological methods inversely up regulated the

expression of several genes involved in lipogenesis mimicking atherosclerosis in rodent models.[55, 59, 61, 68] However, as SREBPs and LXRs are not the focal theme of this review, readers are directed to refer related reviews.[38, 39, 55, 58, 59, 61]

CONCLUSION

The past few years has seen a tremendous rise in obesity among young adolescents and juveniles however, our understanding of the regulating factors of lipogenic genes and lipid metabolism is limited. There has been increasing evidences highlighting the importance of miRNAs in post-transcriptional gene silencing in human disorders like obesity atherosclerosis, NAFLD, NASH and ageing. Micro RNAs are tiny but mighty regulators of gene expression in health and disease hence may be utilized beneficially in targeted therapeutics. This review highlights the recent advances in functional regulation of lipid homeostasis by miRNAs and their potential molecular targets such as SREBPs, LXRs and mRNAs as attractive targets for developing personalized medicine for treating several metabolic disorders. Implications of altered levels of miRNAs like miR-33a,b; miR-122, miR-378, miR-370 and miR-27 has been already utilized as potential targets in several metabolic disorders. Further, elucidating the functional role of miRNAs in human metabolic disorders will shift the paradigm of our knowledge on regulation of gene expression and gene silencing in health and disease.

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REFERENCES

1. Bell CG, Walley AJ, Froguel P. The genetics of human obesity. Nat. Rev. Genet. 2005; 6(3): 221-234.
2. Ogunbode AM, Ladipo MMA, Ajayi IO, Fatiregun AA. Obesity: An emerging disease. Nig. J. Clin. Pract. 2011; 4: 390-394.
3. Ambika GU, Sanjay K, Garg MK. Preventing obesity in India: weighing the options. Ind. J. Endocr. Metabol. 2012; 16: 4-6.
4. Ishola AG. Obesity: As a disease. *Nigerian Clin. Rev. Jour*. 2008; 1: 5-12.
5. Ruth JF Loos. Recent progress in the genetics of common obesity. Br. J. Clin. Pharm. 2009; 68(6): 811-829.
6. Justin David C, Lucumi E, Myers MC, Napper A, Hama K, Farber SA. et al. Identification of novel inhibitors of dietary lipid absorption using zebrafish. PLoS One 2010; 5: e12386.
7. Justin David C. High Cholesterol Diet (HCD) induces Non-Alcoholic Fatty Liver disease (NAFLD) in zebrafish. Eur. J Biomed. Pharm. Sci. 2016a; 3(6): 431-435.
8. Wardle J, Johnson F. Weight and dieting: Examining levels of concern in British adults. Intl. J. Obesity. 2002; 26: 1144-1149.
9. Chhatwal J, Verma M, Kaur Riar S. Obesity among pre-adolescent and adolescents of a developing country. Asia Pac. J Clin. Nutr. 2004; 13(3): 231-235.
10. Kalra S, Unnikrishnan AG. Obesity in India: The weight of the nation. J Med. Nutr. Nutraceut. 2012; 1: 37-41.
11. Ritesh Singh. Childhood obesity: An epidemic in waiting? Intl. J Med. Pub. Health. 2013; 3(1): 2-7.
12. Manu Raj, Krishna Kumar R. Obesity in children and adolescents. Ind. J Med. Res. 2010; 132: 598-607.
13. Azinge EC. Obesity and its implications in 30 Nigerian Patients in Lagos. Niger. Quart. J. Hosp. Med. 1997; 7: 49-52.
14. Oyekan AO. Genetics of food intake, body weight and obesity-role of leptin. Niger. Med. Pract. 2005; 47: 108-112.
15. Song Y, Cone RD. Creation of a genetic model of obesity in a teleost. FASEB J. 2007; 21: 2042-2049.
16. Raghow R, Yellaturu C, Deng X, Park EA, Elam MB. SREBPS: the cross roads of physiological and pathological homeostasis. Tr. Endocr. Met. 2008; 19: 65-73.
17. Schlegel A. Studying non-alcoholic fatty liver disease with zebrafish: a confluence of optics, genetics and physiology. Cell Mol. Life. Sci. 2012; 69: 3953-3961.
18. Justin David C, Vimal P, Rajapriya R, Veena VV, Pandian TJ. Preliminary investigations on generating gnotobiotic zebrafish. Intl. J. Adv. Sci. Engg. 2016a; 2: 138-140.
19. Gerhard GS, Xin C, Craig WG, Genevieve MG, Peter B, Petrick AT et al. Next-generation sequence analysis of genes associated with obesity and non-alcoholic fatty liver disease-related cirrhosis in extreme obesity. Hum. Hered. 2013; 75: 144-151.
20. Xin X, Zhang Y, Liu X, Xin H, Cao Y. and M. Geng. Micro RNA in hepatic fibrosis and cirrhosis. Front. Biosci. 2014; 19: 1418-1424.
21. Justin David C, Rajapriya R, Veena V, G Kumaresan. High Cholesterol diet induces obesity in zebrafish. Intl. J Adv. Sci. Engg. 2016b. (in Press).
22. Justin David C. Preliminary investigations on heart regeneration in obese zebrafish. Eur. J. Biomed. Phar. 2016c. (in Press).
23. Tessitore A, Cicciarelli G, Del Vecchio F, Gaggiano A, Verzella D, M. Fischietti et al. MicroRNA expression analysis in high fat diet-induced NAFLD-NASH,-HCC progression: study on C5&BL/6J mice. BMC Cancer, 2016c; 16: 3-14. DOI: 10.1186/s.

24. Van Amelsvoort L, Schouten E and F. Kok. Duration of shift work related to body mass index and waist to hip ratio. Intl. J. Obes. Relat. Metab. Disord. 1999; 23: 973-978.
25. Bray MS, Young ME. Circadian rhythms in the development of obesity: potential role for the circadian clock within the adipocyte. Obes. Rev. 2006; 8: 169-181.
26. Akhilesh BR, John O'Neil S. Health clocks, healthy body, healthy mind. Cell. 2009; 19: 36-29.
27. Anate M, Olatinwo AW, Omensina AP. Obesity and overview. *West African J. Med.* 1998; 17: 248-254.
28. Akira Kohsaka, Joseph Bass. A sense of time: how molecular clocks organize metabolism. Tr. Endocrinol. Metab. 2006; 18(1): 4-11.
29. Oren F. Circadian rhythms and obesity in mammals. Intl. Schl. Res. Net. Obesity. 2012; ID 437198: 1-12.
30. Manolio TA. Genome wide association studies and assessment of the risk of disease. N. Engl. J Med. 2010; 363: 166-176.
31. Cecil JE, Travendale R, Watt P, Hetherington MM, Palmer CA. An obesity associated FTO gene variant and increased energy intake in children. N. Engl. J Med. 2010; 359: 2558-2566.
32. Rankinen T, Zuberi A, Chagnon YC, Wiesnagel SJ, Argyropuolous G, Walts B. et al. The human obesity gene map: the 2005 update. Obesity Res. 2006; 14(4): 529-644.
33. Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD. et al. Genome wide association (GWA) study for early onset extreme obesity support the role of fat mass and obesity associated gene (FTO) variants. PLoS ONE. 2007; 2: e1361.
34. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM. et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007; 316: 889-894.
35. Chu CY, Chen CF, Rajendran RS, Shen CN, Chen TH, Yen CC. et al. Overexpression of *Akt1* enhances adipogenesis and lead to lipoma formation in zebrafish. PLoS ONE, 2012; 7: e36474.
36. Seth A, Stemple DL, Barroso I. The emerging use of zebrafish to model metabolic disease. Dis. Mod. Mech. 2013; 6: 1080-1088.
37. Antonio JV, Robert VV, Mercedes J, Ariel W, Walter F, Jose FC. et al. Peroxisome –Proliferator activated receptor gene expression in human tissues; effects of obesity, weight loss and regulation by insulin and glucocorticoids. J. Clin. Invest. 1997; 99: 2416-2422.
38. Osborne TF. Sterol regulatory element binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J. Biol. Chem. 2000; 275: 32379-32382.
39. Shimano H. Sterol regulatory element binding proteins (SREBPs): transcriptional regulators of lipid synthesis. Prog. Lipid Res. 2001; 40: 439-452.
40. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with anti-sense complementarity to Lin-14. Cell. 1993; 75: 843-854.
41. Ambros V. MicroRNA pathways in flies and worms; growth, death, fat, stress and timing. Cell. 2003; 113: 673-676.
42. Ambros V. The functions of animal MicroRNAs. Nature. 2004; 431: 350-355.
43. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136: 215-233.
44. Filipowicz W, Bhattacharya SN, Sonenberg N. Mechanisms of posttranscriptional regulation by microRNAs: are the answers in sight. Nat. Rev. Gen. 2008; 9: 102-114.
45. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism and functions. Cell. 2004; 281-297.
46. Betel D, Wilson M, Gabow A, Marks DS, Sander C. The microRNA.org resource: targets and expression. Nucl. Acid. Res. 2008; 36: D149-D153.
47. Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. Nat. Rev. Mol. Cell. Biol. 2012; 13: 239-250.
48. Szabo G, Bala S. Micro RNAs in liver disease. Nat. Rev. Gastroent. Hepatol. 2013; 10: 542-552.
49. Bhatia H, Verma G, Datta M. miR-107 orchestrates ER stress induction and lipid accumulation by post-transcriptional regulation of fatty acid synthase in hepatocytes. Biochim. Biophys. Acta. 2014; 1839: 334-343.
50. Gori M, Arciello M, C. Balsano. Micro RNAs in non-alcoholic fatty liver disease: novel biomarkers and prognostic tools during the transition from steatosis to hepato-carcinoma. Biomed. Res. Intl. 2014; 741465. DOI. 10.1155/2014/741465.
51. Gerhard GS, Distefano JK. Micro RNAs in the development of non-alcoholic fatty liver disease. W. J. Hepatol. 2015; 27(2): 226-234.
52. Alvarez ML, DiStefano JK. Towards microRNA-based therapeutics for diabetic nephropathy. Diabetol. 2013; 56: 444-456.
53. Zhang Y, Cheng X, Lu Z, Wang J, Chen et al. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. Diab. Res. Clin. Pract. 2013; 99: 327-334.
54. Wightman B, Ha I, Ruvkun G. Post-transcriptional regulation of the hetero-chronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans.* Cell. 1993; 75: 855-862.
55. Goldstein JL, Russel A, DeBose, B, Brown M. Protein sensors for membrane sterols. Cell, 2006; 124: 35-46.
56. Stephane G, Y-Hua T, Ronald Khan C. Developmental origin of fat: Tracking obesity to its source. Cell. 2007; 131: 242-256.
57. Sun L, Huang Ming X, Marcelo AM, Ryan A, Bingbing Y, Shilpa M. et al. MiR 193B-365, a brown fat enriched microRNA cluster, is essential for brown fat differentiation. Nat. Cell. Biol. 2011; 13: 958-965.

58. Brown MS, Goldstein JL. The SREBP pathway; regulation of cholesterol metabolism by proteolysis of a membrane bound transcription factor. Cell. 1997; 89: 331-340.
59. Bradley MN, Hong C, Chen M, Joseph SB, Wilpitz DC, Wang X. et al. Ligand activation of LXR beta reverses atherosclerosis and cellular cholesterol overload in mice lacking LXR alpha and apoE. J. Clin. Invest. 2007; 17: 2337-2346.
60. Farber SA, Pack M, Ho SY, Johnson ID, Wagne DS, Dosch R. et al. Genetic analysis of digestive physiology using fluorescent phospholipid reporters. Science. 2001; 292: 1385-1388.
61. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 2002; 109: 1125-1131.
62. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr. Biol. 2002; 12: 735-739.
63. Esau C, Kang X, Peralta E, Hanson E, Marcusson EG, Ravicahndran LV. et al. MicroRNA-143 regulates adipocyte differentiation. J. Biol. Chem. 2004; 279: 52361-52365.
64. Esau C, Davis S, Murray SF, Yu X, Pandey M, Watts L. et al. miR-122 regulation of lipid metabolism revealed by in-vivo antisense targeting. Cell. Metab. 2006; 3: 87-98.
65. Ramsay RR, Gandour RD. Selective modulation of carnitine long chain acyl transferase activities. Kinetics, inhibitors, and active sites of COT, CPT-II. Adv. Exp. Med. Biol. 1999; 466: 103-109.
66. Iliopoulos D, Drosatos K, Hiyama Y, Goldberg IJ, Zanis VI. MicroRNA-370 controls the expression of microRNA-122 and CPT-1 alpha and affects lipid metabolism. J. lipid. Res. 2010; 51: 1513-1523.
67. Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N. et al. miR-33 contribute to the regulation of cholesterol homeostasis. Science. 2010; 328: 1570-1573.
68. Horie T, Nishino T, Baba O, Kuwabara Y, Nakao T, Nishiga M. et al. MicroRNA -33b knock-in mice for an intron of sterol regulatory element-binding factor 1 (Srebf1) exhibit reduced HDL-C- *in vivo*. Sci. Rep. 2014; 16: 5312. (10.1038/srep05312).
69. Marquart TJ, Allen RM, Ory DS, Baldan A. miR-33 links SREBP2 induction to repression of sterol transporters. Proc. Natl. Acad. Sci. 2010; 107: 1228-12232.
70. Voet D, Voet J. Biochemistry. 2010 (4th ed). John Wiley and Sons publishers. New York, USA. 1288pp.
71. Michishita E, McCord RA, Berber E, Kioi M, Padilla NH, Damian M. et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature. 2008; 452: 492-496.
72. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG., Tuschi T, Manoharan M. et al. Silencing of miRNAs *in vivo* with 'antagomirs'. Nature. 2005; 438: 685-689.
73. Gerin I, Bommer GT, McCoin CS, Sousa KM, Krishnan V, MacDougald OA. Roles for miRNA -378/378* in adipocyte gene expression and lipogenesis. Amer. J. Phy. Endocr. Met. 2010; 299: E198-E206.
74. Nakanishi N, Nakagawa Y, Tokushige N, Aoki N, Matsuzaka T, Ishii K. et al. The up-regulation of microRNA -335 is associated with lipid metabolism in liver and white adipose tissue of genetically obese mice. Biochem. Biophys. Res. Comm. 2009; 385: 492-496.
75. Clader J. The discovery of Ezetimibe: a view from outside the receptor. J Med. Chem. 2004; 47: 1l9.
76. Davis HR, Altmann SW. Niemann-Pick C1 like 1 (NPC1L1) an intestinal sterol transporter. Biochim. Biophy. Acta. 2009; 1791: 679-683.
77. Altmann SW, Davis HR, Zhu L, Yao X, Hoos LM, Tetzloff G. et al. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Science. 2004; 303: 1201-1204.
78. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE. et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science. 2010; 328: 1566-1569.
79. Sharifian A, Farahani S, Pasalar P, Gharavi M, Aminian O. Shift work as an oxidative stressor. J Circ. Rhythms. 2005; 3: 15-17.
80. Lefterova MI, Lazar M. New developments in adipogenesis. Trends in Endocr. Met. 2009; 20: 107-114.
81. Romaoa JM, Jin W, Dodson MV, Hausman GJ, Moore SS, Guan LL. MicroRNA regulation in mammalian adipogenesis. Exp. Biol. Med. 2011; 236: 997-1004.
82. Asnani A. Peterson RT. Zebrafish as a tool to identify novel therapies for human cardiovascular disease. Dis. Mod. Mech. 2014; 7: 763-767.
83. Blackburn JS, Langenau M. Zebrafish as a model to assess cancer heterogeneity, progression and relapse. Dis. Mod. Mech. 2014; 7: 7755-7762.
84. Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans.* Science. 2001; 294: 862-864.
85. Chen PY, Manninga, H, Slanchev K, Chien M, Russo JJ, Ju J. et al. The developmental miRNA profiles of zebrafish as determined by small RNA cloning. Genes Dev. 2005; 19: 1288-1293.
86. Tsang J, Zhu J, Van Oudenaarden A. MicroRNA mediated feedback and feed-forward loops are recurrent network motifs in mammals. Mol. Cell. 2007; 26: 753-767.
87. Wienholds E, Koudiis MJ, Van Eden FJ, Cuppen E, Plasterk RH. The microRNA producing enzyme Dicer1 is essential for zebrafish development. Nat. Genet. 2003; 35: 217-218.
88. Wienholds E, Kloosterman WP, Misaka E, Alvarez Saaverdra E, Berezikov E, de Bruijn E. et al.

MicroRNA expression in zebrafish embryonic development. Science. 2005; 309: 310-311.
89. Kloosterman P, Florian A Steiner, Eugene B, Ewart DB, Jose Van De B. et al. Cloning and expression of new microRNAs from zebrafish. Nucl. Acid. Res. 2006; 34(9): 2558-2569.
90. Angulo P. Obesity and non-alcoholic fatty liver disease. Nutr. Rev. 2007; 65: 557-563.
91. Jones GS. The MicroRNA Registry. Nucl. Acid. Res. 2004; 32: D109-111.
92. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S. et al. Micro RNAs regulate brain morphogenesis in zebrafish. Science. 2005; 308: 833-838.
93. Westerfield M. The zebrafish book: A guide for the laboratory use of zebrafish (*Danio rerio*). University of Oregon Press. 1995; 355pp.
94. Santoriello C, Zon LI. Hooked! Modelling human disease in zebrafish. J Clin. Invest. 2012; 122(7): 2337-2343.
95. Taylor AM, Zon LI. Modelling diamond black fan anaemia in the zebrafish. Sem. Haem. 2011; 48(2): 81-88.
96. Semova, I, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, Rawls JF. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Metab. 2012; 12: 277-288.